Exploration of Learning and Memorizing Ability of OSAS rat with Neuroinflammation

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Research

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Abstract

Background

Obstructive sleep apnea syndrome (OSAS) is a respiratory disorder during sleep that has become a significant public health problem over time. OSAS patients elicited the frontal cortex neuron injury, leading to cognitive dysfunction, a significant and extraordinary complication of OSAS patients. Learning and memory testing is critical when assessing potential therapeutic strategies and the effectiveness of treatments to manage OSAS. The objective of this article was to explore the relationship between inflammation and cognitive dysfunction in OSAS.

Methods

Selected male rats were 3-months-old and with a similar weight (mean, 200 ± 10 g) were assigned to the model group (n = 30) and the control group (n = 30). The rats in the OSAS model group were injected with 0.1 ml sodium hyaluronate solution into the upper respiratory tract at the junction between the hard and soft palate. The model and normal rats were compared using tests of Morris Water Maze. The tissue sections were stained by hematoxylin eosin(HE) and immunohistochemically(IHC).

Results

Tests of training test and spatial probe test proved significant differences between 2、4、6 week's OSAS rats over all swims, $p < 0.05$. Histopathology showed cortical laminar oedema with vacuolation ,irregular arrangement, disorganization, rods of the nerve cells were identified, astrocyte activation and reactive gliosis ,abundant astrocyte. And IHC staining showed Iba1-positive cells increased as compared to control group.

Conclusion

We suggest assessing spatial and related forms of learning and memory in OSAS rat by the new statistical method. Microglial and astrocyte activation is involved in OSAS-induced changes in inflammatory molecules, neurogenesis, and spatial memory.

1. Introduction

Obstructive sleep apnea syndrome (OSAS) is a respiratory disorder during sleep, characterized by repeated narrowing and collapse of the pharyngeal airway during sleep, episodes of complete or partial pharyngeal obstruction and plus increases in respiratory effort resulting in intermittent hypoxia (IH)and hypercapnia and sleep fragmentation [6, 30].OSAS recurrent arousals and increase in breath efforts, leading to secondary sympathetic activation, oxidative stress and systemic inflammation. .Associated

complications include cardiovascular disease (coronary artery disease, heart failure, atrial fibrillation, hypertension, and stroke[26, 22, 16],metabolic dysfunction (dyslipidemia and diabetes mellitus), and neurocognitive impairment[14]. Contribute to an overall increase in mortality from related complications [24, 46, 4].Previous studies have revealed that intermittent hypoxia (IH) conditions in OSAS patients elicited brain neuron injury (especially in the hippocampus and frontal cortex) [29, 5, 47],leading to cognitive deficit, a significant and extraordinary complication of OSAS patients. The metabolic and vascular consequences, in turn, increase the severity of OSAS and the risk of both cognitive impairment and dementia and potentially bring forward the age of onset of mild cognitive impairment and Alzheimer's disease (AD), form a vicious circle[9]. The prevalence rate of OSAS in adults is increasing[38, 41],and the number of road traffic and workplace accidents caused by OSAS combined with cognitive dysfunction ,has an adverse impact on patients' quality of life, learning and work efficiency, and health care utilization[15, 23].In children with OSAS, cognitive dysfunction is still an important manifestation, but its underlying pathological mechanism remains unclear[7]. Cognitive functions encompass the broad domains of attention, memory, executive function, visuospatial/constructional abilities, processing speed(cognitive speed) and language (both expressive and receptive). As a whole, negative cognitive effects of OSAS are most likely in the domains of attention/vigilance, verbal and visual delayed long-term memory, visuospatial/constructional abilities, and executive dysfunction[10, 34, 43]. Intermittent hypoxia, as often accompanies obstructive events during sleep, could also be an important contributor to cognitive dysfunction in OSAS[3, 13]. Experimental research and neuroimaging studies show effects of intermittent hypoxia on sleepiness, memory and executive dysfunction. In individuals with OSAS, such changes have been attributed to reduced cell neurogenesis and density of the hippocampus[17, 50, 29], the frontal cortex and generalized grey matter [35, 2, 39, 11].The abnormal changes of gray matter (GM) and white matter may be the important pathological basis of OSAS leading to neurocognitive dysfunction. Numerous studies have shown that GM changes in the frontal cortex are one of the causes of impaired memory and executive function [42, 45, 28]. Therefore the presence of OSAS could be seen as a factor which expedites the process of brain aging by increasing the susceptibility of specific cerebral structures to clinical and pathological occurrences. Increased oxidative stress is associated with chronic intermittent hypoxia-mediated brain cortical neuronal cell apoptosis in a mouse model of sleep apnea. This, in turn, leads to dysfunction of prefrontal regions of the brain cortex[48, 5].

Based on the above studies, a hypothesis is proposed that the changes in frontal cortex and the subcortical structures could be interpreted as enlargement or hypertrophy involving reactive or maladaptive mechanisms, such as cerebral edema, neuronal branching, inflammation, glial activation, or even accumulative Aβ deposition, while atrophy indicates chronic neuronal stimulation Therefore, the purpose of this study was to evaluate spatial learning and memory in OSAS rats using morris water maze(MWM) after the establishment of an OSAS rat model[27] [40, 8] and to observe whether morphological changes in the frontal cortex were involved in reactivity or adaptation-related pathological changes.

2. Materials And Methods

2.1 Animals

Sprague-Dawley rats were purchased from the Animal Experimental Center of Kunming Medical University. The selected male rats were 3-months-old and with a similar weight (mean, 200 ± 10 g), The rats were fed with standard fodder and with food and water freely available in a controlled environment with a constant temperature of 20 ~ 25°C and a 12/12-hr light/dark cycle and were randomly assigned to the model group (n = 30) and the control group (n = 30). Sodium hyaluronate and other chemical reagents were purchased from xiao lai ke mao in China, if not otherwise stated. The independent Ethics Committee of Kunming Medical University approved the study before it began. all experiments were performed in accordance with National Institute of Health Guidelines for the Care and Use of Laboratory Animals

2.2 Surgical Method

Rats in the control group were not treated. Rats in the OSAS model group were given an intraperitoneal injection of 3.6%chloralic hydras(7ml/kg). Following anesthesia, 0.1 ml of sodium hyaluronate gel (15 mg/mL) was injected into the junction between the soft and hard palate[27]. Surgical rats were randomly assigned to the OSAS group, n = 10 for all the groups. The two groups of rats were reared separately with the same feeding conditions, to avoid the influence of other factors on the experimental results.

2.3 Morris Water Maze

Morris water maze was used to assess the spatial learning and memory of rats. consisted of a 1.6-m diameter pool, which was divided into four quadrants (AQ1,OQ□TQ and AQ2)The pool was surrounded by light blue curtains with attached different shapes (such as triangle, square, circle, and pentagon) □these remained in the same position throughout the training and testing periods.. The tank was suffused with the 27-cm-deep water level, which was mixed with 30 ml of ink to obscure visual cues. The temperature of the water was maintained at 23°C–24°C. A platform 12 cm in diameter was hidden 1–2 cm below the surface of the water. The experimental room was soundproof and without direct light. The test consisted of acquisition training test and spatial probe test.

2.3.1 Training test

Briefly, rats were first trained to find a hidden platform in the target quadrant that was submerged in 1–2 cm of water by using a stationary array of cues outside the pool. All testing was conducted at roughly the same time each day in order to minimize variability in performance due to time of day. Acquisition training in the hidden platform was conducted for 5 d consecutively including 20 trials. A trial began by placing the mouse on the platform for 15 s to allow orientation to extra-maze cues. In each trial, the rat was placed into the pool at one of the four possible locations (randomly ordered). After orientation, mice were gently lowered tail-first into the pool facing the wall at one of three positions, each at the center of the wall of a different quadrant not housing the platform. All rats were given 90 s free time to find the hidden platform, and the time spent to reach the escape platform was defined as escape latency. If the rat did not find the platform within 90s, it was gently guided by the observer to the platform for 15s. After

removal from the pool, mice were manually dried with a terrycloth towel and placed in a warming cage (consisting of a heating pad underneath the cage) for at least 5 min before returning to the home cage. Mice were visually inspected to ensure thorough dryness.

2.3.2 Spatial Probe Test

After rest for 1 d, a 90-s probe test was conducted in which the platform was removed. Both during the training and test period, the swimming pathways, percentage of time spent in the target quadrant, platform area crossings, and swimming speed were recorded and calculated using a computer software (XR-XM101,X-maze). The resultant behavioral data were statistically analyzed as described below.

3. Histopathological Evalution

Ten percent of neutral formalin-fixed tissues were embedded in paraffin. Coronal sections were then cut, and stained with hematoxylin-eosin. Random histological images were recorded at magnifications under an Leica DM400B light microscope (Leica ,Germany).

4. Immunohistochemically Analysis

Brain tissue in the hippocampus was fixed in 10% formalin solution and embedded in paraffin. To evaluate histopathological changes in the frontal cortex, 5μm sections were stained with Iba1. Random histological images were recorded at magnifications under an Leica DM400B light microscope (Leica ,Germany). Mean of IOD analysis for the Iba1-positive microglia was performed using Image-Pro Plus 6.0 software.

5. Statistical Analyse

We encoded the accelerated failure time(AFT) survival model to analyze the latency data of morris water maze evasion based on the recommendations of Clark R. Andersen and his paper [1]. We performed the AFT modeling using R's survreg function and RStudio(RStudio Inc.,Boston□USA)statistical analysis, otherwise as described in the paper (Survival/Graph). This approach is commonly referred to as "survival modeling" although in the context of MWM the event of interest is reaching the submerged platform, rather than mortality. Datasets used are included in Supplemental Materials. We adopt Marianne et al [32] propose here a new way to analyze MWM probe test data from the standard reference memory task of the MWM that accurately and simultaneously describes the four variables of time spent in the quadrants and allows to extract more information from the same experiments than the currently used method.□Application of the likelihood-ratio test based on the Dirichlet distribution and Perspectives using Bayesian inference□.Marianne et al provide a package □https://github.com/xuod/dirichlet□contains a python module called dirichlet which can be used with python online or locally, or with R to show how to use the dirichlet module to perform the uniformity test and produce plots with test data and/or your data. Data for Iba1-positive microglial cells comparison was performed with one-way analysis of variance (ANOVA), followed by the LSD and S-N-K multiple comparisons test Which were analyzed by GraphPad

Prism 5.0(GraphPad Software□San Diego□CA□USA). The level of statistical significance was set at $p < 0.05$.

6. Results

6.1 The results of training test

In the MWM task, the animal is required to find a hidden platform to escape from swimming in a pool of water. To accomplish this task, the animal forms a “spatial orientation map” in the brain using visual stimuli from extra-maze cues in the testing room. During training, learning is assessed by the amount of time elapsed before the animal climbs onto the platform to escape the water (escape latency) and by the percentage of time or path length spent in the quadrant housing the platform (target quadrant).A time-to-event AFT model was used to relate the swim duration (latency 90 s) to find the submerged platform (event) to swim day (days 1, 2, 3, 4, 5), swim per day (swims 1, 2, 3, 4), group (control vs OSAS).

The time-to-event AFT model showed significant differences between 2□4□6 week’s OSAS rats over all swims on the everyday, $p < 0.001$, as summarized in Table 1, Table 2, Table 3and Fig. 2. The Fig. 2 shows a consistent trend of lower probability of reaching the platform over time for the OSAS group in comparison with the control group. It also shows the expected trend in increased probability of finding the platform over time both over multiple swims per day and between days. And showed significant differences between OSAS and control group on the same week, p□0.001.In addition, the mean escape latency of 6 week’s OSAS group was significantly increased compared with that of the control group.

6.2 The result of spatial probe test

After the training test, 90-s probe test was conducted in which the platform was removed. In the Fig. 3, each column represents a sample and each color represents a quadrant. Mean values for the fraction of time spent in each quadrant is represented by a dotted line and the error bars on the means are approximated with the inverse Fisher information. For OSAS rat the fraction of time spent in each quadrant is approximately similar leading to a uniform distribution whereas for control rat the

time spent in the target quadrant is significantly higher leading to a non-uniform distribution. And showed significant differences between OSAS and control group on the same week, p□0.05. Corner plot representing constraints on the mean fractions of time m’s for the two data sets control rat (blue) and OSAS rat (green). The diagonal plots show the marginal distributions of m’s (with shaded 68% confidence interval) and off-diagonal plots show the two-dimensional distributions of pairs of these variables (inner and outer contours represent the 68% and 95% confidence levels). The black dashed lines represent the case of uniformity (25%) and the red lines correspond to equal time spent in both considered quadrant[32]. Constraints on m1 (leftmost column) indicate that control rats favor the target quadrant.

7. Effects On Histopathology

Hematoxylin–eosin (HE) staining revealed that rats in control group had clear layers of brain tissue structures, stained evenly and with a regular cell shape, compared with rats in OSAS group. Astrocytes were considered to be of the pale glial nuclei showing a vesicular nucleus with at least one basophilic nucleolus were visible. In 2w's OSAS group, the right cortical pyramidal layer astrocytes is mild vacuolation of internal capsule and major rami. And the left frontal cortex showed cortical laminar oedema with vacuolation ,irregular arrangement, disorganization, rods of the nerve cells were identified, astrocyte activation and reactive gliosis ,abundant astrocyte(Fig. 5). These evidence shows that the histopathological change of frontal cortex is astrocyte hyperplastic inflammatory response.

8. Expression Of The Iba1-positive Microglia

Except the right frontal cortex in 6w's group(Fig. 7B). .Mean of IOD analysis for the Iba1-positive microglia was significant differences between OSAS and control group on the same week, p□0.05(Fig. 7A).which confirmed the promoted effect of OSAS on microglia activation.

9. Discussion

OSAS is a disorder associated with neurocognitive impairment. However, the mechanisms leading to cognitive deficits in OSAS remain controversial. In this study, a rat model of partial pharyngeal occlusion was used to simulate chronic intermittent hypoxia in OSAS[49].The highly-interconnected and neurochemically-rich frontal cortex plays a crucial role in the regulation of mood and cognition, domains disrupted in depression and other central nervous system disorders, and it is an important site of action for their therapeutic control [33]. For improving our understanding of the function and dysfunction of the frontal cortex, we found that OSAS resulted in significantly reduced learning and spatial exploration ability in rats by Morris water maze test. HE showed chronic hypoxia changes in the frontal cortex, with rod cells and micro-vacuolar cell formation in the 2-week group, neuronal cell reduction with astrocyte proliferation in the 4-week group, and homogenized changes in the 6-week group. IHC microglia cells were increased and some of them were hyperchromatic. Compared with the control group, the difference of positive cells in the frontal cortex of each OSAS rat was statistically significant. Our research showed that the activation of microglia and astrocyte in the frontal cortex, involving increased cell number or cell density, and morphological alterations.

9.1 OSAS contributed neurocognitive deficit in rats

OSAS can lead to a series of cardiovascular and cerebrovascular problems and associated with a number of adverse health consequences. A growing literature focuses on its neurocognitive correlates. Although research in this field is mixed, multiple studies indicate that OSAS patients show impairment in attention, memory, and executive function.[20] Some researchers argue that intermittent hypoxemia leads to prefrontal cortical degeneration, which could explain the impairment in executive function observed in patients with OSAS[5]. The frontal cortex is an important brain region for central executive function and is the core subcomponent of execution and memory, thirty-nine task-based functional magnetic resonance

imaging (fMRI) studies (697 mild cognitive impairment (MCI) patients and 628 healthy controls) were included in MCI-related meta-analysis while 36 task-based fMRI studies (421 AD patients and 512 healthy controls) were included in AD-related meta-analysis. The meta-analytic results revealed that both MCI-related and AD-related hyperactivation the frontal lobe relative to healthy controls [19, 25].Damage to the frontal cortex results in impaired executive function, learning and memory performance.

9.2 Changed in Histopathology

Oxidative stress-induced glial cells activation, neuroinflammation and mitochondrial dysfunction lead to various molecular events in brain neurons causing neuronal cell death in the frontal cortex of OSAS rats[12].Key to this immune to brain communication is that glia, microglia, and astrocytes, interpret and propagate inflammatory signals in the brain that influence physiological and behavioral responses[36]. Activated microglia proliferate and accumulate in areas presenting high densities of apoptotic neurons to facilitate neuronal turnover during developmental cell death[44].As microglia are the brain's innate immune effector cells, dysregulation of microglial behavior appears to be a critical component of this progression, brain microglia become dysregulated with age and cause chronic neuroinflammation [31].There is key differential expression and timing of cytokine expression of microglia and astrocytes that are interpreted to indicate sequential activation and evidence of dynamic communication between two cell types. Activated astrocytes produce many regulatory factors that may influence CNS immunity and provide negative feedback to activated microglia[36]. Immunocytochemically positive for Iba1 protein were the microglia,a study indicated that localisation of Iba1 protein is restricted to microglia both in vitro and in vivo, and that Iba1 protein plays a role in regulating the function of microglia, especially in the activated microglia[18].

OSAS intermittent hypoxia in oxidative stress activated microglia and astrocytes, chronic hypoxic cerebral cortex damage early have cortical laminar oedema with vacuolation ,irregular arrangement, disorganization, rods of the nerve cells were identified, astrocyte activation and reactive gliosis ,abundant astrocyte[37].Our study showed the microglia ,astrocyte activation and reactive gliosis. The increased evidence collected supports that OSAS should be viewed as low-grade chronic inflammatory diseases and the existence of inflammation can be considered a potential contributing factor to OSAS pathophysiology and comorbidity.[21]. The important role played by inflammation in OSAS related cognitive dysfunction. CIH characterized in OSAS leads to peripheral inflammation which access the CNS through BBB or via the stimulation of vagal afferents. The high level of inflammation in the CNS further upregulates glial cells (microglia and astrocyte) activity, inducing and aggravating the neuroinflammatory reaction. Meanwhile, CIH could directly activate microglia and astrocyte, prompting the release of inflammatory cytokines in the CNS. The frontal cortex is the most sensitive to hypoxia, so this area is involved in most neurocognitive dysfunction. IH may affect microglia directly or indirectly through peripheral or other CNS cells, require an understanding of how intermittent hypoxia promotes neuroinflammation, neuronal death and cognitive deficits.

10. Conclusion

We encode a new statistical method of survival model to analyze Morris Water Maze spatial probe test data and suggest assessing spatial and related forms of learning and memory in OSAS rat by the new statistical method. A better understanding of the role of microglia and astrocytes in the regulation of OSAS pathology is needed as this could pave the way for new therapeutic strategies.

11. R Code(Survival/graph)

```
library(ggpubr)  #package for combining plots in a single image
library(ggplot) #package for plotting
library(survival) # package for modeling
library(hommel) # for the hommel p values
test = read.csv("~/final.csv") #This is my version of the excel file
# This is the main Idea, we will run a survival regression then make a prediction of what is
# the mean given our model for each level of probability plus the confidence intervals.
# Then we are going to make a table for the difference in means with it's respective calculations
#### See the AIC to test what distribution is better ####
# first we list all the possible distributions and then make a loop printing the AIC
dist = c("weibull", "exponential", "gaussian", "logistic","lognormal", "loglogistic")
for (i in 1:length(dist)){
m= survreg(Surv(Result)~ factor(round)+factor(control)+factor(day), dist=dist[i], data = test)
print(paste0("AIC ", dist[i], ": ", extractAIC(m)[2]))
}
#this indicates that we should use logistic, but in the next lines we use loglogistic following the paper
#### WRITING THE MODEL ####
# we have to make the outcome (latency time, a survival variable, but we assume that all of
# the rat survived because we don't have other data), and we explain the difference in the
# latency by the model, the day, and the round of swiming if you have wich rats did
# reach the platform in (it should be a dichotomous variable) change the model like this:
# model1 = survreg(Surv(Result, NEW_VARIABLE)~factor(control)+factor(round)+factor(day),
#                  dist="loglogistic", data = test)

model1 = survreg(Surv(Result)~factor(control)+factor(round)+factor(day),
                 dist="loglogistic", data = test)
# we are going to make 20 plots by hand because it's literally 7 minutes (I measured it)
# we could autumate it eval on the line plotPart54 I don't like evals but it's the only
# way of dinamucally generatte the plots inside of a loop using the function eval
```

```
# it should be a loop inside of a loop where  the last line is
# eval(parse(text=paste0("plotPart",i,j,"= ggplot(plotData, aes(x = fit, y = Probability,
# color = factor(control)) )+ geom_line(aes(x = fit))+
# geom_ribbon( aes(xmin = lwr, xmax = upr, color = factor(control)), alpha=0.2, linetype = 0)+
# coord_cartesian(xlim =c(0, 91), ylim = c(0, 100)) +
# scale_colour_discrete(name  =NULL,
#                       breaks=c(0, 1),
#                       labels=c('OSAS', 'Control'))+
#   ggtitle(title) +
#  labs(y='Probability (%)', x = 'Escape latency(s)')")))


i= 1 # day
j= 1 # round
# we first make the prediction of the control
pred1 <-as.data.frame(predict(model1, newdata=list(round=j, control=1, day =
i),type="quantile",p=seq(.01,.99,by=.01), se=TRUE))
pred1$Probability = 1:99 # we create the probability variable
pred1$lwr = pred1$fit-1.96*pred1$se.fit #lower level confidence interval. if want to
# change meassure change the z level that is 1.96. As you know the usuals are 1.645 1.96
# 2.575 being 90%, 95%, and 99% confidence
pred1$upr = pred1$fit+1.96*pred1$se.fit # same that aboove
pred1$control=1 # we created a dummy variable to indicate this probabilitys are for treatement
#the next lines are exacly the same than above but for the OSAS, as you notice the name
# of the variable change but the code is the same
pred0 <-as.data.frame(predict(model1, newdata=list(round=j, control=0, day =
i),type="quantile",p=seq(.01,.99,by=.01), se=TRUE))
pred0$Probability = 1:99
pred0$lwr = pred0$fit-1.96*pred0$se.fit
pred0$upr = pred0$fit+1.96*pred0$se.fit
pred0$control=0
```

```
# we create a single dataframe with both predictions
plotData = rbind(pred0, pred1)
title = paste0("Day ",i,", Swim ", j) # this is the title of each individual plot
plotPart54 = ggplot(plotData, aes(x = fit, y = Probability, color = factor(control)) )+
geom_line(aes(x = fit))+ # we plot the central line
# the next line geom_ribon creates the confidence interval shadow
geom_ribbon( aes(xmin = lwr, xmax = upr, color = factor(control)), alpha=0.2, linetype = 0)+
# we want to show the plot only to 90 seconds, and here it is
coord_cartesian(xlim =c(0, 90), ylim = c(0, 100)) +
# we want the labels be OSAS and Control instead of 0 and 1 and no title
scale_colour_discrete(name  =NULL,
breaks=c(0, 1),
labels=c("OSAS", "Control"))+
# add the title to the plot
ggtitle(title) +
# change the axis labels
labs(y="Probability (%)", x = "Escape latency(s)")
# if you make it in a loop this is wehre it should end
# the next line just add all the plots into a single one with a common legend
figure <- ggarrange(plotPart11, plotPart12, plotPart13, plotPart14,
                    plotPart21, plotPart22, plotPart23, plotPart24,
                    plotPart31, plotPart32, plotPart33, plotPart34,
                    plotPart41, plotPart42, plotPart43, plotPart44,
                    plotPart51, plotPart52, plotPart53, plotPart54,
                    ncol = 4, nrow = 5,
                    common.legend = TRUE, legend = "bottom")
figure

#### CREATE THE TABLE ####
```

```
# We first create a matrix of the size we want
result = matrix(data = NA, nrow = 20, ncol = 9)
result[,1] <- rep(1:5, each=4) # populate the days column repeating 1 to 5 each number 4 times
result[,2] <- rep(1:4) # populate the round column repeating the sequence 1 to 4
#loop to though the matrix
for (i in 1:NROW(result)){
# predict the expected mean for the given swim day and round for control
pred1 <-as.data.frame(predict(model1, newdata=list(round=result[i,2], control=1,
                                        day = result[i,1]),
                              type="quantile",p=.5, se=TRUE))
# predict the expected mean for the given swim day and round for test
   pred0 <-as.data.frame(predict(model1, newdata=list(round=result[i,2], control=0,
                                        day = result[i,1]),
                              type="quantile",p=.5, se=TRUE))
   result[i,3] = pred0$fit-pred1$fit # calculate the difference in means
   result[i,4] = sqrt((pred0$se.fit^2)/10+(pred1$se.fit^2)/10) #calculate the standard error of the
difference
   result[i,5] = pred0$fit/pred1$fit # ratio of the expected mean
   result[i,6] = result[i,3]-1.96*result[i,4] # Lower bound confidence interval of the difference
   result[i,7] = result[i,3]+1.96*result[i,4] # Upper bound confidence interval of the difference
   result[i,8] = pnorm((0-result[i,3])/result[i,4]) # pvalue
   result[i,9] = hommel(result[i,8], simes=TRUE)@adjusted # hommel p value
 }
result = as.data.frame(result) # we transform it into a dataframe so we can name the columns
#with the array names
names = c("Day","Swim","estimate","SE","exp(estimate)","CI95Min","CI95Max","p-value","Hommel")
colnames(result) = names # we just set the columns names as we want
write.csv(result, "Results.csv") # export it to csv
```

Declarations

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The independent Ethics Committee of Kunming Medical University approved the study before it began. All experiments were performed in accordance with National Institute of Health Guidelines for the Care and Use of Laboratory Animals.

CONSENT FOR PUBLICATION

All authors have read and approved this version of the article for publication.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed in this study are available from the corresponding author on reasonable request.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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AUTHOR CONTRIBUTIONS

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Tables

Table1. The AFT model-adjusted differences between 2wOSAS and 2wControl, by day and swim per day

Day	Swim	estimate	SE	exp(estim	CI95Min	CI95Max	p-value	Hommel
1	1	30.05264	4.363267	1.491698	21.50064	38.60465	2.84E-12	2.84E-12
1	2	26.2668	3.908122	1.491698	18.60688	33.92672	9.02E-12	9.02E-12
1	3	13.795	2.346524	1.491698	9.195812	18.39419	2.06E-09	2.06E-09
1	4	20.79745	3.181057	1.491698	14.56258	27.03232	3.12E-11	3.12E-11
2	1	15.1531	2.397187	1.491698	10.45461	19.85158	1.30E-10	1.30E-10
2	2	13.2442	2.083179	1.491698	9.161174	17.32724	1.02E-10	1.02E-10
2	3	6.955693	1.268622	1.491698	4.469193	9.442193	2.09E-08	2.09E-08
2	4	10.48646	1.77367	1.491698	7.010063	13.96285	1.69E-09	1.69E-09
3	1	19.69739	2.958205	1.491698	13.8993	25.49547	1.38E-11	1.38E-11
3	2	17.21603	2.621068	1.491698	12.07874	22.35333	2.54E-11	2.54E-11
3	3	9.041648	1.648758	1.491698	5.810082	12.27321	2.08E-08	2.08E-08
3	4	13.63126	2.243625	1.491698	9.233754	18.02876	6.18E-10	6.18E-10
4	1	21.0507	3.562559	1.491698	14.06809	28.03332	1.72E-09	1.72E-09
4	2	18.39887	3.124289	1.491698	12.27526	24.52248	1.94E-09	1.94E-09
4	3	9.662858	1.936693	1.491698	5.866941	13.45878	3.03E-07	3.03E-07
4	4	14.5678	2.702353	1.491698	9.271188	19.86441	3.51E-08	3.51E-08
5	1	23.08242	3.361394	1.491698	16.49409	29.67075	3.28E-12	3.28E-12
5	2	20.17464	2.976739	1.491698	14.34023	26.00905	6.12E-12	6.12E-12
5	3	10.59547	1.885018	1.491698	6.900838	14.29011	9.50E-09	9.50E-09
5	4	15.97382	2.50279	1.491698	11.06835	20.87928	8.72E-11	8.72E-11

Table 2 The AFT model-adjusted differences between 4wOSAS and 4wControl, by day and swim per day

Day	Swim	estimate	SE	exp(estim	CI95Min	CI95Max	p-value	Hommel
1	1	28.87483	2.880597	1.506984	23.22886	34.5208	5.98E-24	5.98E-24
1	2	25.41915	2.604223	1.506984	20.31488	30.52343	8.30E-23	8.30E-23
1	3	19.70879	2.109918	1.506984	15.57335	23.84423	4.77E-21	4.77E-21
1	4	22.24955	2.286149	1.506984	17.7687	26.7304	1.10E-22	1.10E-22
2	1	27.42632	2.949666	1.506984	21.64497	33.20766	7.15E-21	7.15E-21
2	2	24.144	2.641292	1.506984	18.96706	29.32093	3.09E-20	3.09E-20
2	3	18.72009	2.158101	1.506984	14.49021	22.94997	2.08E-18	2.08E-18
2	4	21.1334	2.322615	1.506984	16.58107	25.68572	4.56E-20	4.56E-20
3	1	28.03228	2.787162	1.506984	22.56944	33.49512	4.25E-24	4.25E-24
3	2	24.67744	2.473937	1.506984	19.82852	29.52635	9.81E-24	9.81E-24
3	3	19.1337	2.036491	1.506984	15.14217	23.12522	2.85E-21	2.85E-21
3	4	21.60032	2.177454	1.506984	17.33252	25.86813	1.70E-23	1.70E-23
4	1	34.52849	3.5953	1.506984	27.4817	41.57528	3.85E-22	3.85E-22
4	2	30.3962	3.158581	1.506984	24.20538	36.58702	3.19E-22	3.19E-22
4	3	23.56775	2.566408	1.506984	18.53759	28.59791	2.09E-20	2.09E-20
4	4	26.60599	2.780105	1.506984	21.15699	32.055	5.34E-22	5.34E-22
5	1	21.85383	2.277074	1.506984	17.39077	26.31689	4.10E-22	4.10E-22
5	2	19.23841	2.031796	1.506984	15.25609	23.22073	1.42E-21	1.42E-21
5	3	14.91654	1.797729	1.506984	11.39299	18.44009	5.32E-17	5.32E-17
5	4	16.83951	1.918213	1.506984	13.07981	20.59921	8.27E-19	8.27E-19

Table 3. The AFT model-adjusted differences between 6wOSAS and 6wControl, by day and swim per day

Day	Swim	estimate	SE	exp(estin	CI95Min	CI95Max	p-value	Hommel
1	1	56.00288	2.012066	3.121406	52.05923	59.94653	8.53E-171	8.53E-171
1	2	50.99396	1.881854	3.121406	47.30553	54.6824	5.24E-162	5.24E-162
1	3	29.43342	1.24148	3.121406	27.00012	31.86672	1.48E-124	1.48E-124
1	4	49.21409	1.790935	3.121406	45.70386	52.72432	1.54E-166	1.54E-166
2	1	77.30545	2.688221	3.121406	72.03654	82.57436	3.69E-182	3.69E-182
2	2	70.39123	2.430703	3.121406	65.62705	75.15541	1.07E-184	1.07E-184
2	3	40.62941	1.630495	3.121406	37.43364	43.82518	2.35E-137	2.35E-137
2	4	67.93432	2.401918	3.121406	63.22656	72.64208	2.77E-176	2.77E-176
3	1	63.52093	2.153628	3.121406	59.29982	67.74204	1.68E-191	1.68E-191
3	2	57.8396	1.972387	3.121406	53.97372	61.70548	2.51E-189	2.51E-189
3	3	33.38468	1.357281	3.121406	30.72441	36.04495	6.84E-134	6.84E-134
3	4	55.82079	1.895287	3.121406	52.10603	59.53555	5.86E-191	5.86E-191
4	1	69.68338	2.311755	3.121406	65.15234	74.21442	6.61E-200	6.61E-200
4	2	63.45088	2.103469	3.121406	59.32808	67.57368	3.42E-200	3.42E-200
4	3	36.62348	1.445195	3.121406	33.7909	39.45606	5.57E-142	5.57E-142
4	4	61.23622	2.04037	3.121406	57.23709	65.23534	3.39E-198	3.39E-198
5	1	64.49176	2.119437	3.121406	60.33766	68.64586	1.15E-203	1.15E-203
5	2	58.7236	1.935595	3.121406	54.92983	62.51736	1.77E-202	1.77E-202
5	3	33.89492	1.363512	3.121406	31.22243	36.5674	1.05E-136	1.05E-136
5	4	56.67393	1.865535	3.121406	53.01748	60.33038	5.13E-203	5.13E-203

Annotation:Estimate is the difference in the point prediction at the 50th percentile;
SE is the standard error of the difference;
exp(estimate) is the ratio of OSAS time over Control time;
CI95Min lower bound confidence interval (1.96 standard deviations);
CI95Max upper bound confidence interval (1.96 standard deviations);
p-value simple p value based on Z score;
Hommel p-value corrected for multiple testing.

Figures

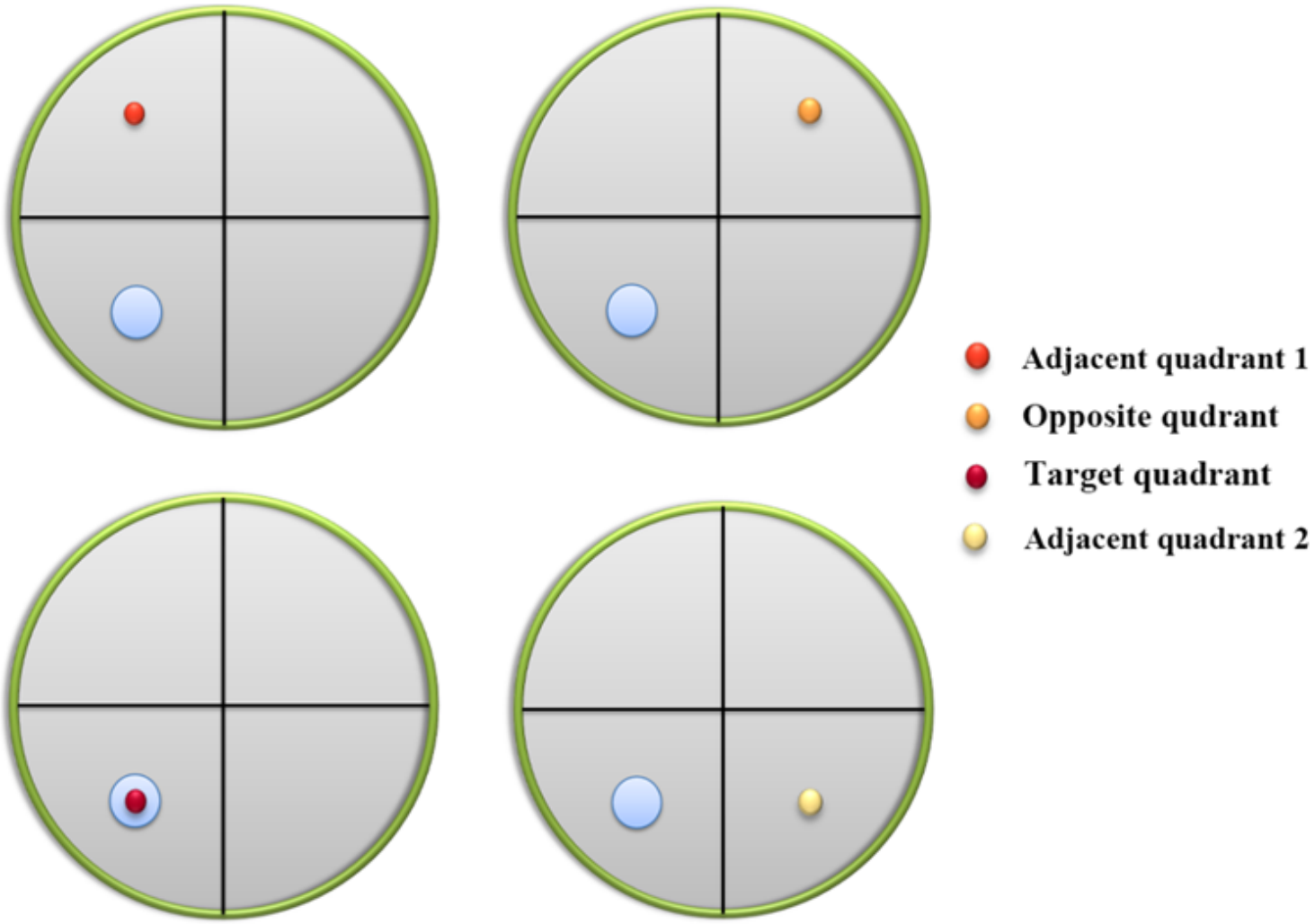

Figure 1

Schematic diagram of MWM. Adjacent quadrant 1(AQ1);Opposite quadrant(OQ);Target quadrant(TQ); Adjacent quadrant 2(AQ2).

Figure 2

Probability of reaching the hidden platform over time for each OSAS group, per predictions from the time-to-event AFT model, by day and swim-per-day.

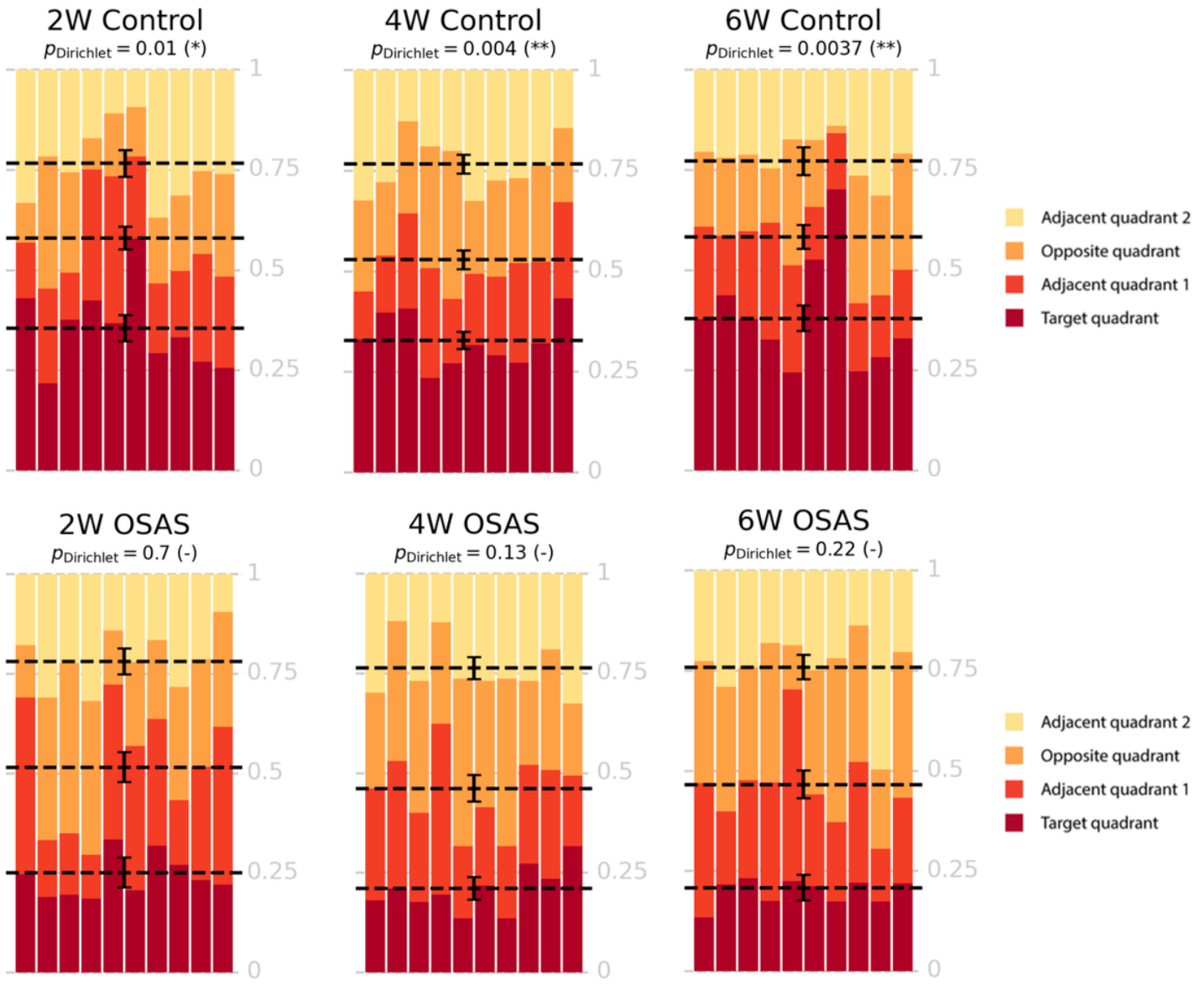

Figure 3

Time spent in the four quadrants by OSAS rats and control rats. The data were expressed as mean ± SEM. *p < 0.05 and ** P <0.01 vs. Control.

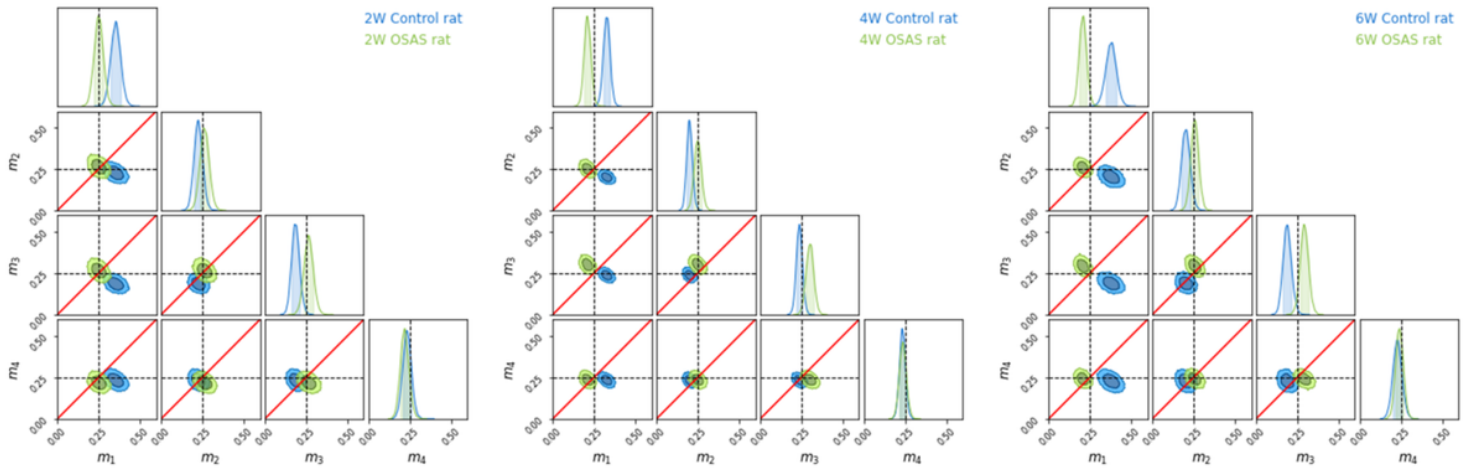

Figure 4

Fraction of time spent in the four quadrants for OSAS rats and control rats in the case of Bayesian inference. m1 represents the target quadrant (TQ).

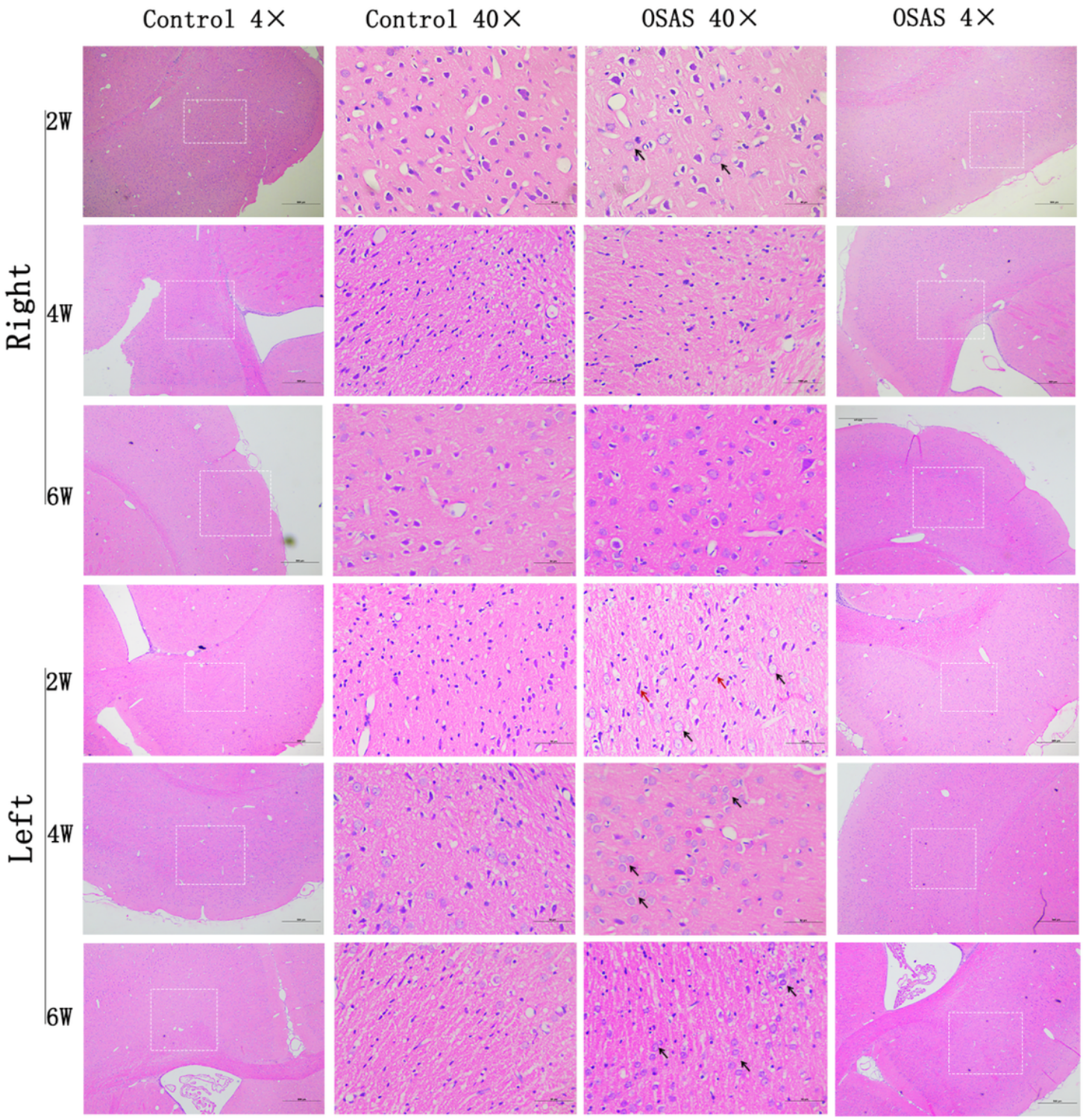

Figure 5

The morphological and structural changes of neurons in OSAS and control groups, astrocytes is mild vacuolation or abundant (black arrows), denatured nerve cells(red arrows). Original magnification = 4×

/40× and scale bar = 500μm/50μm.

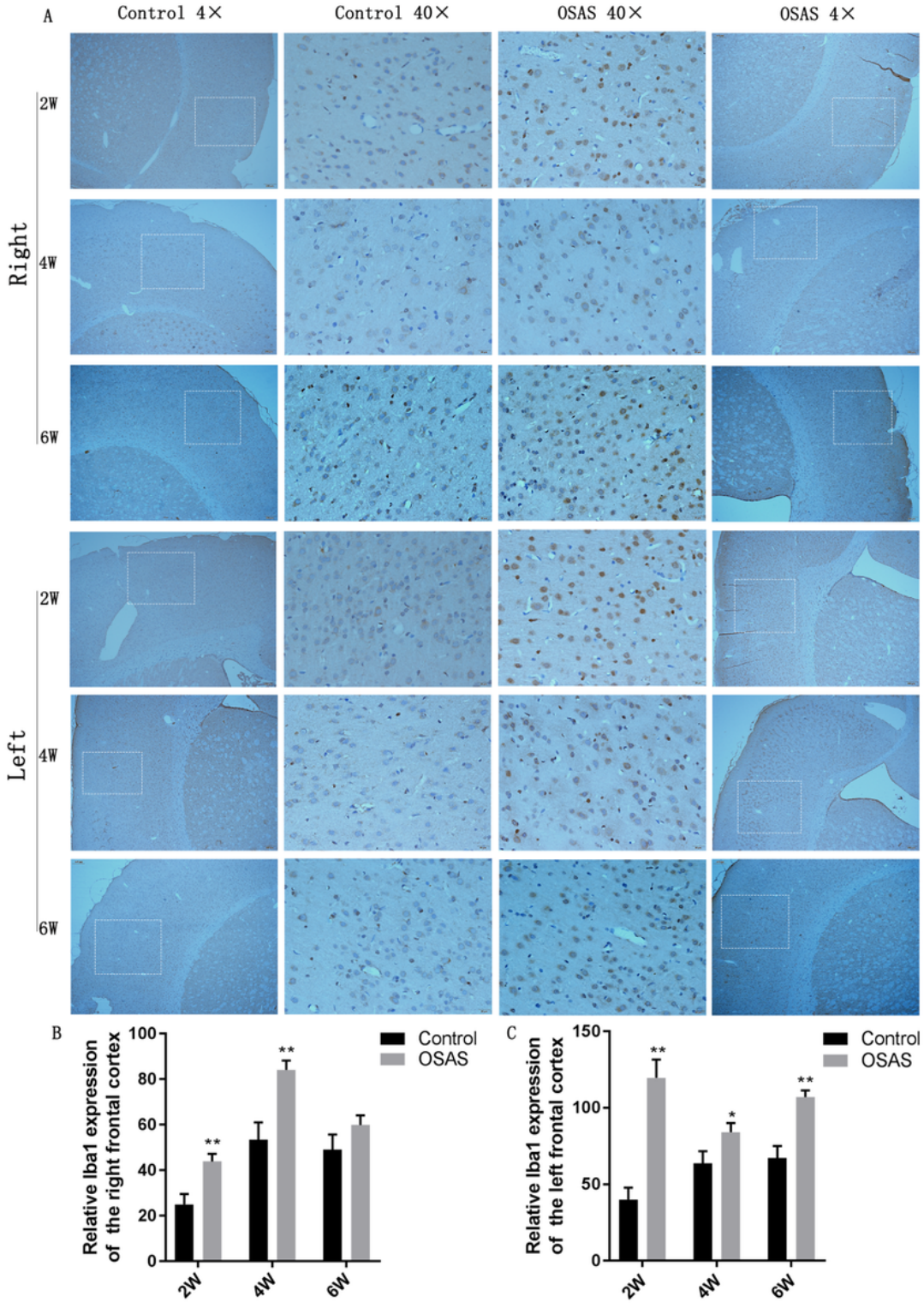

Figure 6

Iba1-positive microglial cells changes of the frontal cortex in OSAS and control groups. Original magnification = 4× /40× and scale bar = 500 μm/50μm.B,C The data were expressed as mean ± SEM and compared using unpaired t-test one-way ANOVA. *p < 0.05 and ** P <0.01 vs. Control.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- final.csv