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Role of GHRH in Sleep and Growth Impairments Induced by Upper Airway Obstruction in Rats

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Abstract

Upper airway obstruction (UAO) can lead to abnormal growth hormone homeostasis and growth retardation by unclear mechanisms. We explored the effect of UAO on hypothalamic growth hormone releasing hormone (GHRH), which has a role in both sleep and growth hormone regulation.

The tracheae of 22-day-old rats were narrowed; UAO and sham-operated animals were sacrificed 16 days post-surgery. To stimulate slow wave sleep and growth hormone secretion, rats were treated with ritanserin (5-HT2 receptor antagonist). Sleep was measured with telemetric system. Hypothalamic GHRH, hypothalamic GHRH and growth hormone receptors, and orexin were analyzed using ELISA, real-time PCR, and Western immunoblot.

UAO decreased hypothalamic GHRH, GHRH and growth hormone receptors levels, while orexin mRNA increased ($p<0.01$). In UAO rats the duration of wakefulness was elevated and the duration of slow wave sleep (SWS), paradoxical sleep, and slow wave activity were reduced ($p<0.001$). Ritanserin alleviated these effects, i.e., normalized hypothalamic GHRH content, decreased wake duration, increased duration and depth of SWS, and attenuated growth impairment ($p<0.001$).

Here we present evidence that growth retardation in UAO is associated with a reduction in hypothalamic GHRH content. Our findings show that the abnormalities in GHRH/ growth hormone axis underlie both growth retardation and SWS disorder UAO.

Keywords: Upper airway loading, growth retardation, growth hormone axis, sleep, rat

Introduction

Children with upper airway obstruction usually suffer from sleep disordered breathing and growth retardation [1–4]. We previously hypothesized that the impairment of growth is related to reduce growth hormone release from the pituitary gland, which normally requires undisturbed slow wave sleep [1]. The release of growth hormone is greatly enhanced during sleep, especially early in the night; this is associated with the appearance of delta waves on electroencephalography characteristic of slow wave sleep [SWS; 5], and increased release of growth hormone releasing hormone (GHRH) in the hypothalamus [6]. The mutual relationships between sleep and GHRH are complex. Central or systemic administration of exogenous GHRH enhances non-rapid eye movement sleep while inhibition of endogenous GHRH secretion suppresses sleep duration and depth [7–9]. Genetic mutations associated with GHRH deficiency lead to reduction of both depth and duration of non-rapid eye movement sleep and to a decrease in growth hormone secretion, resulting in dwarfism [9].

The possibility that GHRH may be affected in children with sleep-disordered breathing emerges from studies showing that both slow wave activity, a quantitative measure for non-rapid eye movement sleep depth, [10] and growth hormone axis are suppressed [1]. Moreover, all these sleep and hormonal disturbances as well as growth retardation are reversed following surgical removal of the upper airway obstruction [1,2,10]. In adult humans, growth hormone and slow wave activity normalized following treatment of sleep-disordered breathing with continuous positive airway pressure [11,12].

Ritanserin, a selective 5-HT2 receptor antagonist, increases endogenous growth hormone release and non-rapid eye movement sleep [13]. The strong association between sleep and growth hormone release raises the possibility that pharmacological agents that

increase non-rapid eye movement sleep can also stimulate growth hormone secretion [13].

We have used chronic upper airway obstruction (UAO) in juvenile rats as a model bearing some features of human sleep disordered breathing and subglottic stenosis [14,15]. In this model, we have shown that UAO causes growth retardation, which is related to an impairment of the global and local growth hormone/insulin-like growth factor-1 axis. In parallel, UAO also fragments non-rapid eye movement sleep [16].

The mechanisms linking UAO with neuroendocrine disturbances, which cause growth retardation and sleep abnormalities, are unknown. In this study, we hypothesized that UAO affects sleep and growth by causing a decrease in hypothalamic GHRH levels. We found that UAO indeed causes depletion of hypothalamic GHRH. We also provide evidence that pharmacological blockade of 5-HT2 receptors could prevent the UAO-induced depletion of the hypothalamic GHRH content, preserve sleep, and attenuate growth retardation.

Methods

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Surgery: Tracheal narrowing surgery (anesthesia tribromoethanol, 200 mg/kg ip) was used to induce UAO in 22-day-old Sprague-Dawley male rats [14,15]. Controls underwent surgery with no tracheal narrowing. Animals were returned to their cages and kept in 12:12 h light-dark cycle, lights on 09:00. On day 9, telemetric transmitters for sleep recording were implanted (see below). Food and water were given *ad libitum.* The study was approved by the Ben-Gurion University of the Negev Animal Use and Care Committee and complied with the American Physiological Society Guidelines.

Experimental schedule: Sleep was recorded on day 15 (baseline) and day 16 following acute administration of high dose ritanserin (Sigma-Aldrich Ltd., Israel), a 5-HT2 receptor antagonist, at 09:00. In separate series of animals, hypothalamic GHRH, growth hormone receptor, GHRH receptor, orexin mRNA and proteins, serum growth hormone and insulin-like growth factor-1 levels, and respiratory parameters were measured on day 15. Chronic ritanserin study: In an additional series of experiments UAO and control rats were treated with a high dose of ritanserin to stimulate slow wave sleep and growth hormone secretion. Animals were injected i.p. with ritanserin (2 mg/kg) or vehicle at lights on for 8 consecutive days [15].

Sleep: Data were collected on day 16 (DSI, St. Paul, Minnesota, US) and sleep signals were scored using software (NeuroScore v. 2.1, DSI) and edited visually. The duration of sleep-wake states were calculated in 1-h time blocks [8] and were categorized as wake, slow wave sleep (SWS) and paradoxical sleep (PS). Power density during the first 3 hours of light was performed separately for each vigilance state [17]. The power density values for 0.5–4.0 Hz were integrated and used as an index of electroencephalogram slow wave activity during non-rapid-eye-movement sleep [10]. The effect of ritanserin on slow wave activity was calculated as the ratio of mean slow wave activity obtained following ritanserin versus slow wave activity obtained at baseline [17].

Hypothalamic mRNA expression: RNA was extracted, and quantitative real time PCR assays were performed [15] for: GHRH, GHRH receptor, growth hormone receptor, orexin, and β-actin. Total RNA from two animals was pooled and assayed as one sample, yielding 8 combined samples in each group.

Proteins were determined by Western immunoblot [14,15] in a subset of 7 sham and 8 UAO rats on day 16.

Immunoassays: Serum growth hormone and insulin-like growth factor-1 concentrations were measured using ELISA kits (DSL, Inc., Webster, TX, USA) on day 16.

Hypothalamic GHRH was determined by ELISA kit (USCN Life Science Inc., Wuhan, China) according to the manufacturer's instructions on day 16.

Data analysis: Significance was analyzed by unpaired *t*-test. One-way analysis of variance was used to determine significance differences in hypothalamic GHRH following administration of ritanserin or vehicle. Two-way analysis of variance for repeated measures was used to determine significance between time and group, or frequency and group, for *post hoc* comparisons by Student–Newman–Kuels test. The null hypotheses were rejected at the 5% level.

Results

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A total of 117 animals were included in this study; the final numbers of animals were 55 and 50 for the UAO and sham control groups, respectively. During surgical procedure mortality rate of the UAO group was 10% and an additional 10% mortality was observed 2–5 days after surgery.

*At days 13-16, the UAO rats' behavior was similar to controls; they explored their cage and engaged in social activity such as grooming. The obstructed animals all demonstrated audible wheezing, especially after activity, but no signs of respiratory distress or gasping at rest were observed among UAO animals. As expected, following tracheal obstruction surgery, there were signs of increased mechanical load, i.e., inspiratory swings in esophageal pressure (ΔPes) more than doubled (*p*=0.03), respiratory rate decreased (*p*<0.001), and tracheal resistance (n=8 in each group) increased by 46% (*p*=0.03) (Table 1E). Arterial blood gases and hemoglobin (n=8 in*

each group) were normal and unchanged between groups (Table 1E). Growth rate was significantly lower in UAO animals compared to controls (n=11 in each group) (Table 2E). Thirteen days post-surgery, food intake was unchanged (148±3.4 gr food/kg and 164±6.9 gr food/kg, respectively, p*=0.1) in the control (n=10) and UAO (n=11) rats. Both the control (n=8) and UAO groups (n=8) exhibited circadian rhythms of body temperature. Mean 12-hr light period body temperature was 37.0±0.03°C and 36.5±0.03°C for the control and UAO rats, respectively, (*p*<0.01). During dark period mean 12-hr body temperature was lower in UAO rats compared with controls (37.5±0.04°C and 37.0±0.04°C, respectively,* p*<0.01).*

Sleep duration: Both the control (n=11) and UAO (n=11) rats exhibited circadian rhythms of wake, SWS, and PS. As expected for nocturnal animals, there was more sleep in the light period than in the dark period (Figure 1). There were, however, several significant differences in sleep between UAO and control groups (Figure 1, Table 1). The obstructed group was awake 36% more during the light period ($p<0.001$) and had 18% less SWS duration during the light period ($p<0.001$). UAO rats had significant elevation of light slow wave sleep (SWS-1) and reduction of deep slow wave sleep (SWS-2), compared with controls during the first 6 hrs of the light period ($p<0.01$). Interestingly, during the dark period UAO animals had 38.3% more SWS than controls ($p<0.001$). Obstructed animals had 41% less PS ($p<0.001$) than controls during both light and dark periods (Figure 1, Table 1).

To determine the total duration of each sleep–wake stage, we quantified the number and length of each sleep stage epoch (Figure 2) for 12-hr light periods. UAO animals had more frequent interrupted sleep compared with controls. The obstructed group had 35% more wake bouts and they were longer by 67% than in controls ($p<0.001$). UAO rats

manifested a 37% reduction in the number of SWS bouts ($p<0.01$) without any change in duration and 50% decrease of bout numbers and duration of PS ($p<0.001$).

Power density analysis: During SWS, the power density of UAO (n=11) rats decreased by 40% at 1.5 Hz compared with controls (n=11) (Figure 1E, $p<0.001$). Spectral analysis of light slow wave sleep and deep slow wave sleep in the UAO group during the first 3 hrs of the light period decreased by 37% and 57% (data are not shown), in the frequency range of 0.5–4 Hz compared with controls, respectively ($p<0.001$). The time course of electroencephalogram slow wave activity during non-rapid eye movement sleep showed a normal pattern in the controls with high slow wave activity values at the beginning of the rest period, followed by a decline towards dark onset, and a gradual increase during the dark period (Figure 3). In contrast, the daily course of electroencephalogram slow wave activity was flat in UAO; there were no significant changes detected in slow wave activity depth across the day ($p<0.001$).

Ritanserin Study: Acute administration of high dose 5-HT2 receptor antagonist, ritanserin, significantly decreased ($p<0.01$) wake duration during lights on in UAO rats (n=10 in each group) to levels statistically similar to those of controls (Figure 4). In both groups ritanserin significantly increased slow wave sleep duration up to 6 hrs following drug administration; this was related to elevation of deep slow wave sleep (SWS-2) duration (Figure 4 and Table 1). Ritanserin increased slow wave activity up to 6 hrs following drug administration in both groups ($p<0.01$, Figure 3B). Confirming earlier reports [15] during chronic ritanserin study, growth gain parameters were about 50% less ($p<0.001$) in UAO (n=12) compared to controls (n=10). Ritanserin partially normalized somatic growth retardation as revealed in tail and tibial lengths, but not in body weight

gain (Figure 5). UAO decreased serum insulin-like growth factor-1 in rats treated with vehicle (700±80 ng/mL) compared to UAO treated with vehicle (550±50 ng/mL) (p=0.01). Ritanserin normalized this effect on serum insulin-like growth factor-1 values (680±90 ng/mL) that was not statistically significant from the control group.

Endocrine analysis was performed in a subset of 10 sham and 12 UAO rats**:** Serum growth hormone (control rats, 101±8 ng/mL and UAO 62±5 ng/mL, p=0.038) and insulin-like growth factor-1 level (control rats, 1400±86 ng/mL and UAO n=9, 870±60 ng/mL, p=0.001) were significantly reduced in UAO. Hypothalamic GHRH mRNA (Figure 6A) and protein (Figure 6B) decreased in UAO animals by 30% (p=0.002) and 25% (p=0.001), respectively. Hypothalamic GHRH receptor protein (Figure 6C) decreased by 72% in UAO animals (p=0.001). Administration of ritanserin, for eight days at lights on, normalized hypothalamic GHRH content in UAO animals (controls administered ritanserin, 1.6±0.29 pg/mg tissue; UAO animals administered ritanserin 1.5±0.19 pg/mg tissue, p=0.327; one way ANOVA), n=10 in each group. Hypothalamic orexin mRNA (Figure 3E) increased in UAO by 73% (p<0.01).

Discussion

We have shown that growth retardation in UAO is associated with reduction of hypothalamic GHRH content. The latter could explain both the abnormal slow wave sleep and impaired growth hormone homeostasis in juvenile UAO rats. Our data suggest that a similar mechanism could be responsible for growth retardation of children suffering from upper airway obstruction without preserved sleep architecture.

It is not clear why growth retardation occurs only in 5% to 25% of children with sleep-disordered breathing [1–4]. Several controversial mechanisms have been suggested to explain this phenomenon: dysphagia and decreased appetite [4], increased work of breathing during sleep [3], and abnormal growth hormone homeostasis [1,2]. In this study, we offer the suggestion of the involvement of impaired growth hormone axis as a major mechanism of growth retardation. In contrast to our UAO rats, children with upper airway obstruction typically do not have an elevated arousal index secondary to obstructive events and have preserved sleep stage durations [19]. Our results may suggest that growth retardation may occur in a subset of children without preserved sleep architecture. We have also shown that pharmacological correction of the abnormalities in GHRH/growth hormone axis may potentially be a promising strategy for therapeutic interventions designed to improve growth in affected children.

The model strength and limitation

Chronic upper airway loading was induced in 22-day-old rats, and animals were followed for two weeks, a period comparable to six months to eight years age range in children. Following surgery, inspiratory swings in esophageal pressure and tracheal resistance increased, consistent with increased airways resistive loading. Previously, we demonstrated that UAO led to decreased respiratory rate and tidal volume accompanied by mild elevation of arterial PCO_2 [15,20]. Because our measurements of Pes were taken under anesthesia, they may underestimate the true efforts of obstruction on intrathoracic pressure. The unchanged PCO_2, reduced respiratory rate, and Pes in the current study suggest that the trachea was not severely obstructed and could be considered typical for mild to moderate respiratory loading. Under these conditions animals were able to maintain normal resting ventilation and arterial PO_2 [14,15,20]. In children, however,

oxygen saturation usually decreases with airway loading during sleep [21]. The UAO model has been used to explore adaptive changes in respiratory system function [14,15,20,22,23]. In this model, both inspiratory and expiratory loading was introduced that may resemble subglottic stenosis in children and be not exclusively sleep related, while in clinical sleep-disordered breathing, airway loading is mainly inspiratory and sleep related [19]. Obstructive sleep apnea is associated with intermittent upper airway obstruction at night, primarily during inspiration. It seems likely that our model also has implications for this condition since, like in sleep apnea, the animals exhibited sleep fragmentation and as in children with sleep apnea, growth retardation.

Ritanserin treatment normalized the growth hormone axis and growth parameters were partially restored. This finding may suggest that, in addition to growth hormone and GHRH, other mechanisms may also be involved in growth retardation in this model. It is possible that there is impairment of total body energy balance related to increased work of breathing [3] and/or increased energy expenditure due to enhanced locomotion activity due to the sleep fragmentation. There is little evidence to support these possibilities, as total body energy in UAO rats during quiet wakefulness was similar to that in controls [14]. Mean locomotor activity in UAO rats was unchanged or even decreased during active and quiet phases of the day, respectively [15]. Finally, protein loss and cachexia may have been induced following increased production of oxygen-derived free radicals and cytokines, as a result of strenuous contractions of respiratory muscles associated with upper airway loading [24]. However, previous studies have not found changes in serum pro-inflammatory factors such as interleukin-6 and tumor necrosis factor-alpha in UAO rats [25].

Upper airway obstruction and **GHRH/GH axis**

Chronic upper airway obstruction inhibited the secretion of hypothalamic GHRH in our model. Hypothalamic GHRH is necessary to maintain GHRH receptor synthesis and both growth hormone and GHRH regulate their own synthesis by negative feedback at the level of the hypothalamus [9,26–28]. In order to better understand the distribution and localization of GHRH and its receptor in specific hypothalamic nuclei, future studies should be done using immunocytochemical staining. In our study PS was significantly reduced in UAO rats. Paradoxical sleep deprivation can significantly decrease GHRH mRNA in the paraventricular and arcuate nuclei [29].

Orexins/hypocretins are neuropeptides, inhibiting GHRH in hypothalamic nuclei involved in non-rapid eye movement and rapid eye movement sleep regulation [30]. In UAO rats, orexin mRNA increased by 73%, possibly as a result of forced physical activity [22] and/or increased awakening [31]. Interestingly, the neuroendocrine effects, which we observed in UAO rats, resemble those seen following prolonged sleep-deprivation/restriction in rats, including decreased body temperature [32], depletion of hypothalamic GHRH content [7,8] and reduction of serum growth [33]. It is possible that sleep fragmentation due to UAO leads to orexin increase and abnormal serotonergic balance, which results in reduced SWS and GHRH [30]. Further studies are needed to explore this issue.

The strong association between non-rapid eye movement sleep and growth hormone release raises the possibility that pharmacological agents that increase non-rapid eye movement sleep can also stimulate growth hormone secretion [13. In the current study hypothalamic GHRH-growth hormone axis and sleep in UAO rats were reversed by administration of ritanserin. Stimulation of growth hormone secretion and promotion of

sleep are two closely interrelated outputs of hypothalamic GHRHergic neurons [9]. As in our study, administration of a high dose of ritanserin has a strong sleep consolidation effect in rats [18] and in humans with preexisting sleep fragmentation [34]. This could provide additional explanation for the improved sleep duration and slow wave activity following ritanserin administration in UAO. Finally, similar to other reports [18,35], the effects of ritanserin on slow wave sleep and wake cycles are limited to the first hours of light onset following drug administration due to its known pharmacokinetics.

We found that decreased GHRH was associated with both sleep and growth impairment. This is consistent with studies showing that sleep duration and depth are suppressed when GHRH is inhibited by means of a competitive GHRH receptor antagonist, activation of the negative feedback in the somatotropic axis, or after somatostatinergic stimulation [7,8]. Slow wave activity is also regulated, in part, via intrinsic cortical GHRH [36]. Slow wave activity increases during wakefulness, due to cortical and motor activities during awakening and slow wave activity declines during sleep [37]. Indeed, consistent with this, our UAO rats spent significantly more time in SWS (see Figure 1 middle panel) during the dark phase and were less active during the dark phase [15]. Further studies are needed to explore to explore the role of cortical GHRH in juvenile UAO rats.
Several possibilities may explain the reduction of PS in our UAO rats. During PS the skeletal muscle force is minimal and insufficient to support ventilation during chronic resistive loading. During PS the arousal threshold to acoustic stimulation is lower [38], resulting in shortening of this stage in our rats. Our UAO animals have lower growth hormone levels. Rats with impairment in GHRH signaling have less PS than normal rats [39].

Conclusion

This study present evidence that growth retardation in UAO is associated with a reduction in hypothalamic GHRH content. Our findings show that the abnormalities in GHRH/ growth hormone axis underlie both growth retardation and SWS disorder UAO.

Acknowledgments

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Figure Legends

Figure 1: Spontaneous sleep in control and UAO rats. Hourly values of wake (W), slow wave sleep (SWS) and paradoxical sleep (PS) are shown. Black horizontal bars represent the light-off (active) period on a 12:12-h cycle with light on at 09:00. UAO had significantly more wake and less SWS and PS than controls during light period. During dark period UAO had significantly more SWS and less PS than controls. Data are from 11 control and 11 UAO rats. # indicates statistically significant ($p<0.001$) difference between the groups. Values are mean ± SEM.

Figure 2: Total number (top) and length (bottom) of each sleep-wake stage during 12-hr light on period. W – wake, SWS – slow wave sleep, PS – paradoxical sleep. # $p<0.01$, Values are mean ± SEM.

Figure 3: (A) Hourly average of electroencephalogram slow wave activity (SWA, integrated power densities in delta range). SWA was significantly lower in UAO rats compared with controls. (B) Effect of ritanserin (2 mg/kg) on SWA calculated as percent baseline values on day 15 (arrow identifies time of injection). Black horizontal bars represent light off on a 12:12-h cycle with light onset 09:00. In panel A, # indicates statistically significant ($p<0.001$) difference between groups. In panel B, # indicates statistically significant ($p<0.01$) differences following ritanserin relative to baseline vehicle values on day 15 in both groups. Values are mean ± SEM.

Figure 4**:** Effect of ritanserin (2 mg/kg) on spontaneous sleep. Hourly values of wake, slow wave sleep (SWS), and paradoxical sleep (PS) are shown; left column control and right column UAO. Baseline - vehicle study (day 15) and ritanserin (2 mg/kg) study day 16. Ritanserin or vehicle was administered at light on (arrow). Black horizontal bars

represent the light-off (active) period on a 12:12-h cycle with light on at 09:00. # Indicates statistically significant (p<0.001) difference between baseline and ritanserin values. Values are mean ± SEM.

Figure 5: Effect of ritanserin on somatic growth parameters. Upper panel body weight gain, middle panel tail length gain, lower panel tibial length gain. Control + vehicle (C); Control + ritanserin (C-Rit); Upper airway obstruction + vehicle (UAO); Upper airway obstruction + ritanserin (UAO-Rit). * Statistically significant, C vs. UAO; # statistically significant, UAO vs. UAO-Rit.

Figure 6: (A) Hypothalamus growth hormone-releasing hormone (GHRH) relative mRNA level determined by real time PCR. (B) Hypothalamus GHRH content, determined by ELISA. (C) Top – representative Western immunoblot analysis of hypothalamus GHRH receptor (GHRHR). Bottom – densitometric analysis of GHRHR protein. # p=0.002, * p = 001.

Table 1: Spontaneous sleep values during 12-hr light on period.

	Control (N=10)		Obstructive (N=10)	
	Baseline	**Ritanserin**	**Baseline**	**Ritanserin**
Wake (%)	38.9 ± 2.3	33.6 ± 2.2	52.9 ± 2.6+	41.1 ± 2.3*
SWS (%)	45.3 ± 1.6	52.0 ± 1.9*	37.1 ± 2.6+	48.7 ± 1.8*
PS (%)	15.7 ± 1.8	14.6 ± 0.8	9.2 ± 1.2+	10.6 ± 1.0*
SWS-1 (%)	31.8 ± 1.0	31.1 ± 0.9	29.1 ±2.2	29.2 ± 1.5
SWS-2 (%)	13.7 ± 1.3	21.0 ± 2.1*	8.4 ± 2.2+	19.0 ± 1.4*

A verage % of time spent in each sleep stage for light period (09:00–21:00). PS – paradoxical sleep; SWS – slow wave sleep; SWS-1 – light slow wave sleep; SWS-2 – deep slow wave sleep (stages 3 and 4 in humans). + $p<0.01$ comparing baseline (vehicle) control with baseline (vehicle) UAO. * $p<0.001$ comparing ritanserin (2 mg/kg) with baseline (vehicle). Significant differences were determined by two-way ANOVA. Values are mean ± SEM.

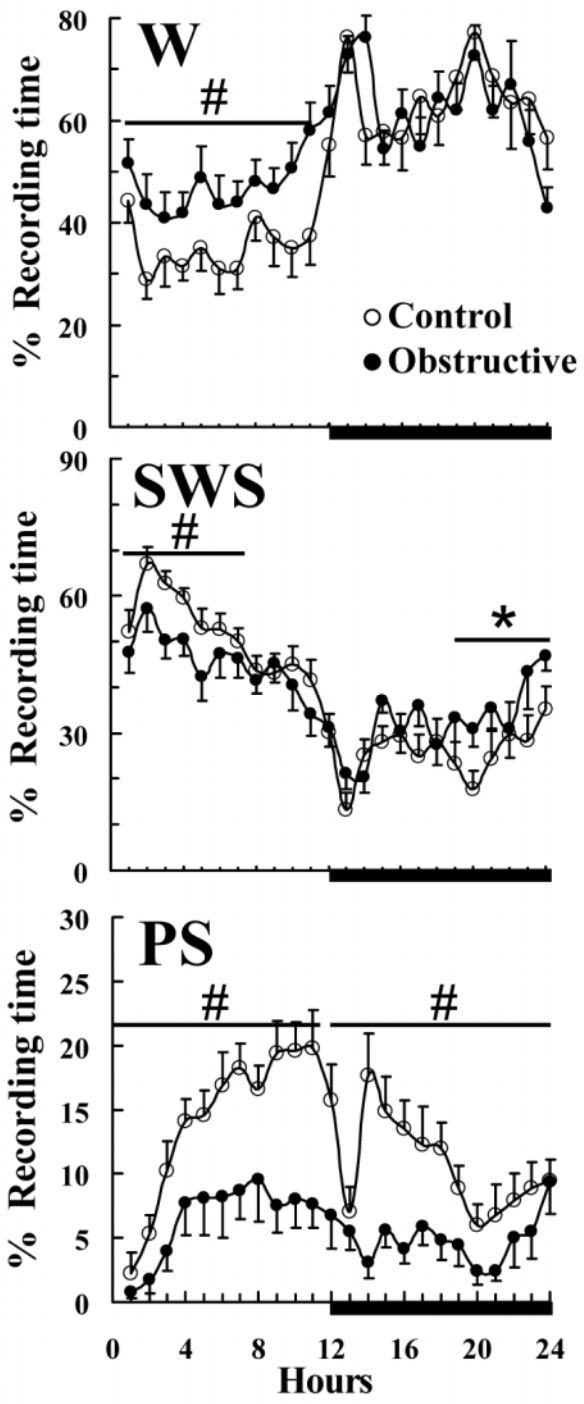
W
#
% Recording time
80
60
40
20
0
Control
Obstructive
SWS
#
*
% Recording time
90
60
30
0
PS
#
#
% Recording time
30
25
20
15
10
5
0
0
4
8
12
16
20
24
Hours

Figure 1

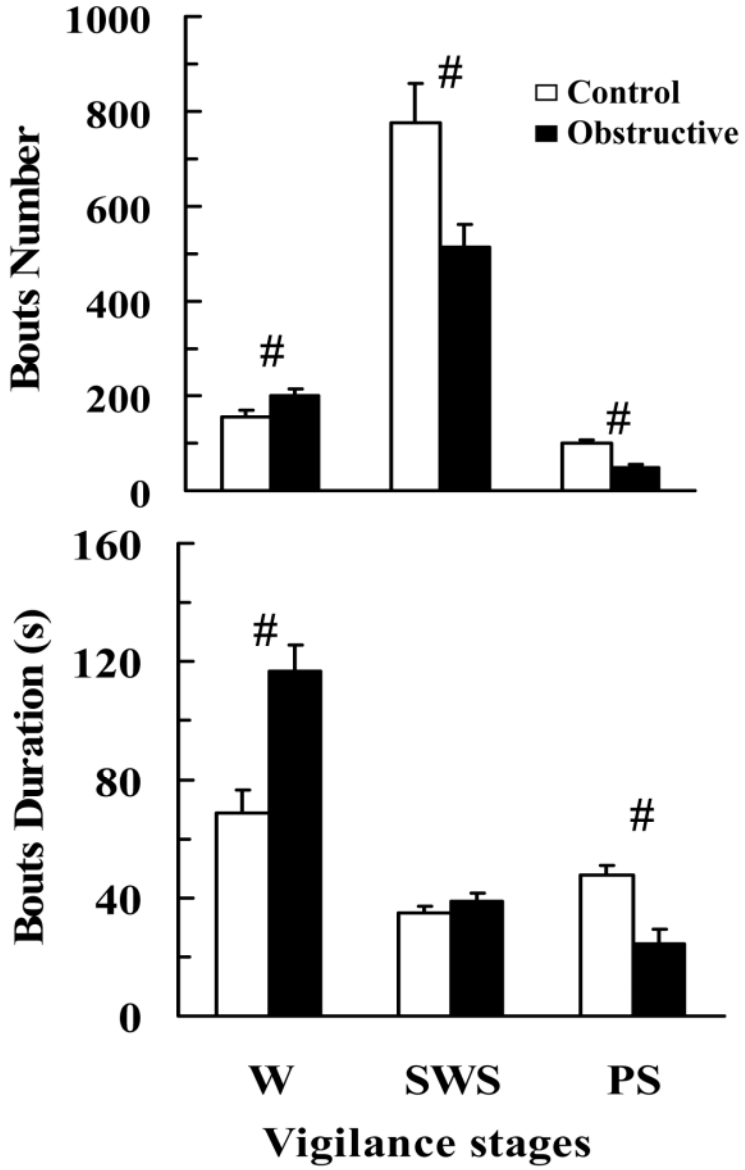
1000
800
600
400
200
0
Bouts Number
#
#
#
Control
Obstructive
160
120
80
40
0
Bouts Duration (s)
#
#
W
SWS
PS
Vigilance stages

Figure 2

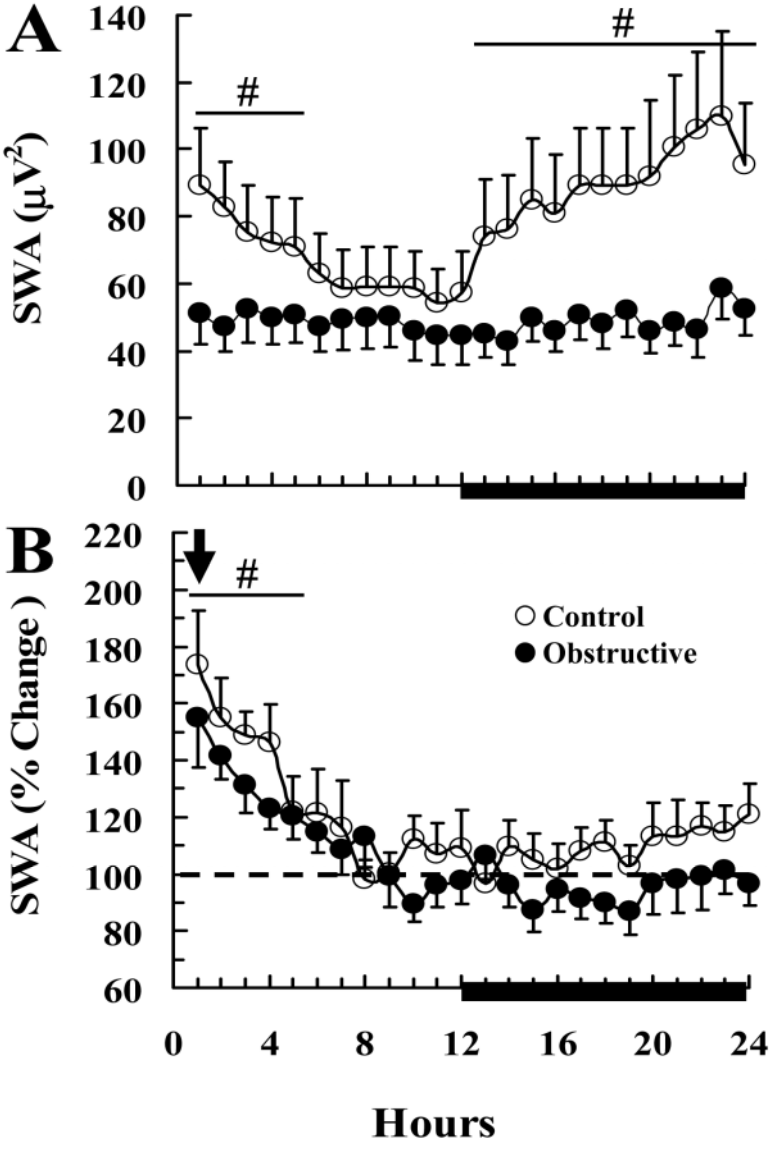
A
B
SWA (μV²)
SWA (% Change)
#
#
#
Control
Obstructive
140
120
100
80
60
40
20
0
220
200
180
160
140
120
100
80
60
0
4
8
12
16
20
24
Hours

Figure 3

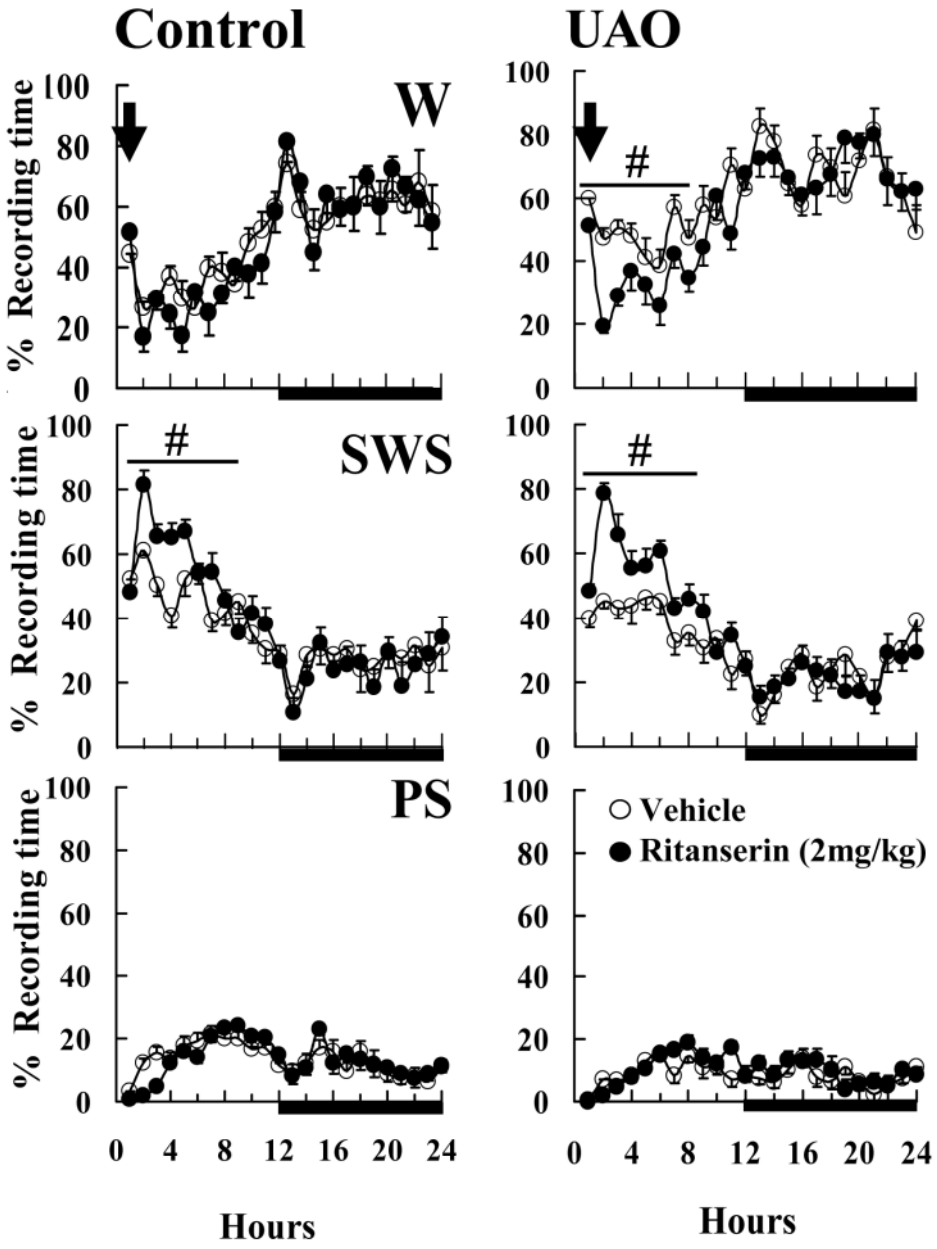
Control
UAO
W
SWS
PS
#
% Recording time
100
80
60
40
20
0
Vehicle
Ritanserin (2mg/kg)
0 4 8 12 16 20 24
Hours

Figure 4

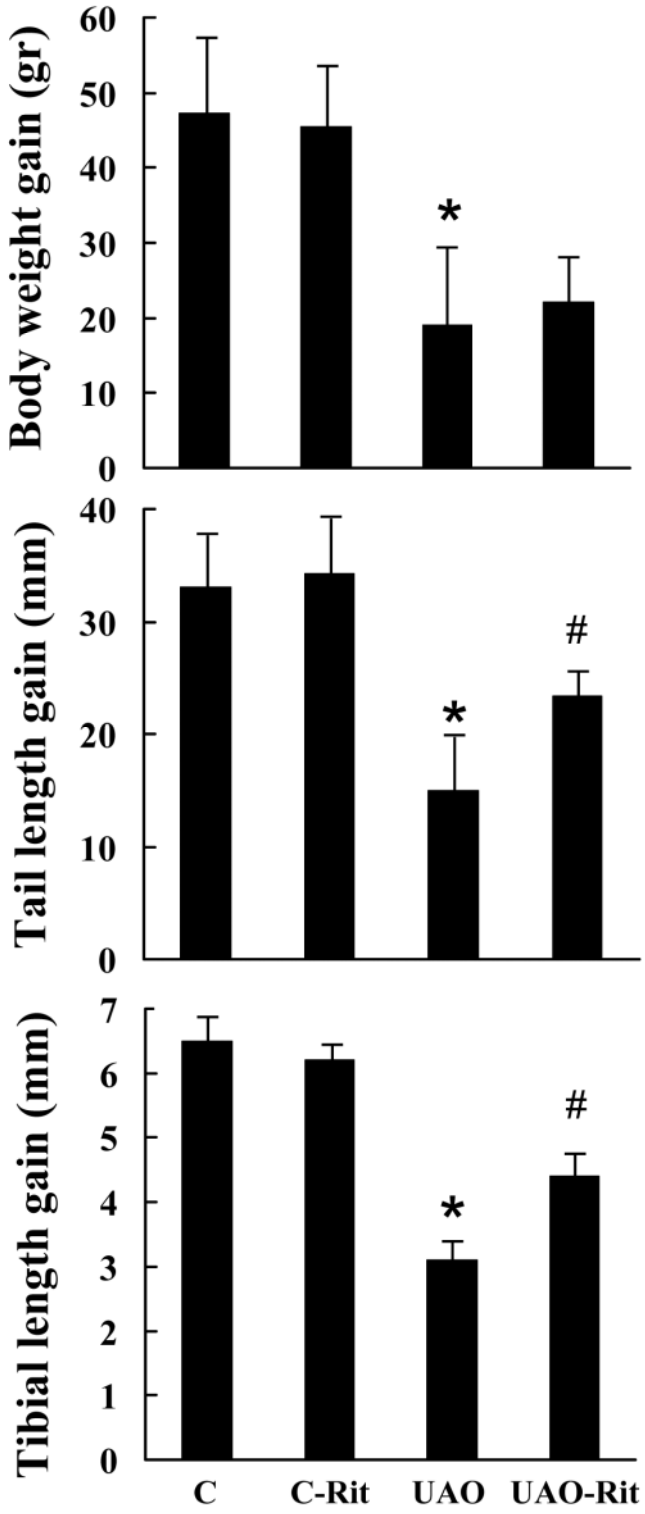
Body weight gain (gr)
60
50
40
30
20
10
0
*
Tail length gain (mm)
40
30
20
10
0
*
#
Tibial length gain (mm)
7
6
5
4
3
2
1
0
*
#
C
C-Rit
UAO
UAO-Rit

Figure 5

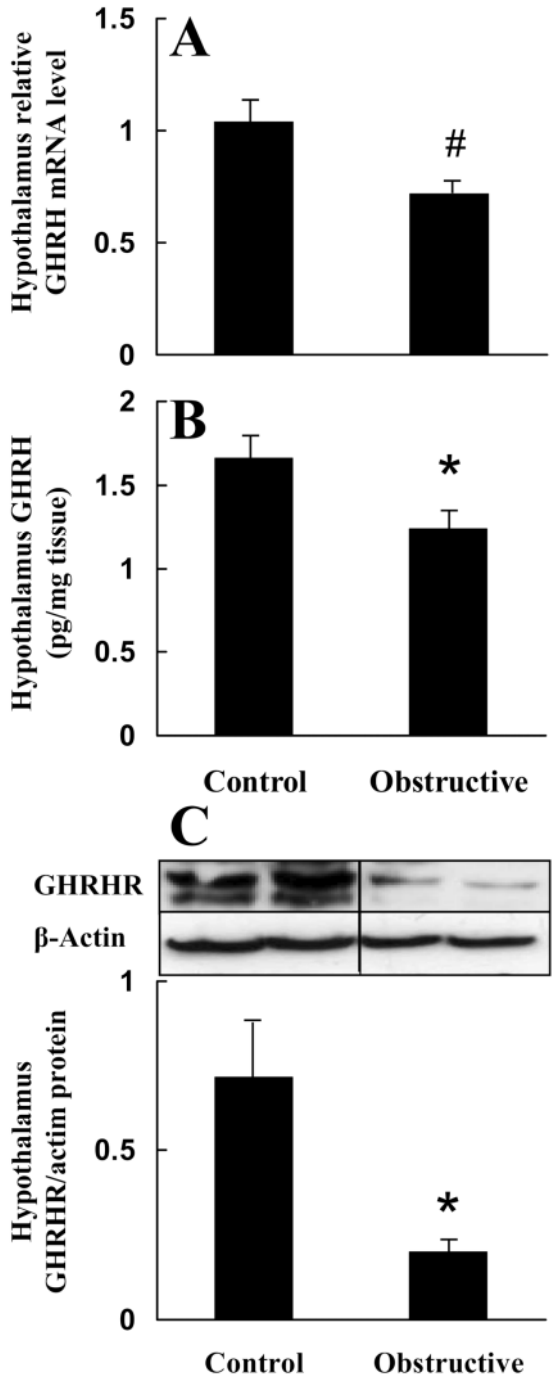
A
Hypothalamus relative GHRH mRNA level
1.5
1
0.5
0
#
B
Hypothalamus GHRH (pg/mg tissue)
2
1.5
1
0.5
0
*
Control
Obstructive
C
GHRHR
β-Actin
Hypothalamus GHRHR/actim protein
1
0.5
0
*
Control
Obstructive

Figure 6