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Spatial learning and memory deficits following exposure to 24 hours of sleep fragmentation or intermittent hypoxia in a rat model of obstructive sleep apnea

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Abstract

Obstructive sleep apnea is primarily characterized by hypoxemia due to frequent apneic episodes and fragmentation of sleep due to the brief arousals that terminate the apneic episodes. Though neurobehavioral deficits frequently accompany sleep apnea, the relative roles of hypoxia versus sleep fragmentation are difficult to separate in apneic patients. Here, we assessed cognitive function as measured by water maze in the Fischer/Brown Norway (FBN) rat, comparing 24 h of sleep interruption (SI) to 24 h of intermittent hypoxia (IH), in order to dissociate their relative contributions to cognitive impairment. For SI, automated treadmills were used to induce brief ambulation in rats every 2 min, either prior to, or after, initial water maze acquisition training. IH was simulated by cycling environmental oxygen levels between 6% and 19% every 2 min, again either prior to, or after, acquisition. 24 h of IH exposure had no significant effect on either acquisition or retention, irrespective of whether IH occurred prior to, or after, acquisition. To replicate previous work, another group of rats, exposed to 3 days of IH (10 h/day) prior to acquisition, had impaired performance during acquisition. A comparison of the 24 h IH and 3 day IH findings suggest that a minimum amount of IH exposure is necessary to produce detectable spatial memory impairments. Although SI before acquisition had no effect on acquisition or later retention of the hidden platform location, SI after acquisition robustly impaired retention, indicating that spatial memory consolidation is more susceptible to the effects of sleep disruption than is the acquisition (learning) of spatial information.

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*Institution where work was performed.

Keywords

sleep apnea; sleep fragmentation; intermittent hypoxia; water maze; spatial memory

1. Introduction

Sleep fragmentation and intermittent hypoxia are primary characteristics of obstructive sleep apnea (OSA), and may lead to the symptoms associated with OSA, including excessive daytime sleepiness and neurocognitive impairments (Verstraeten, 2007). The extent of impairment observed in apneic patients has been correlated with both the severity of sleep fragmentation and the degree of blood gas abnormalities (hypoxemia) (Bedard et al., 1991). It might be intuitively presumed that neurocognitive impairment is due to the reduction of oxygen to the brain (Row, 2007), since acute exposure to low oxygen levels (e.g., at high altitude) can produce cognitive impairments (Maiti et al., 2008). However, a recent study employing functional imaging determined that the working memory deficits exhibited in patients with sleep apnea may be due to sleep fragmentation, not nocturnal hypoxia (Thomas et al., 2005). Although both sleep fragmentation and hypoxemia may contribute to the cognitive deficits seen in sleep apnea, their relative effects can be difficult to determine in OSA clinical populations because sleep fragmentation and intermittent hypoxia occur together.

The excessive daytime sleepiness and neurocognitive impairments which characterize sleep apnea are thought to be linked, perhaps causally, to the frequent arousals and restructuring of sleep caused by sleep fragmentation/interruption (SI), rather than the loss of sleep time, *per se*. Thus, the SI model employed in the present experiment was designed to mimic the disturbances of continuity, deep sleep, and REM sleep seen in sleep apnea, or in a night of experimental sleep fragmentation (Roehrs et al., 1985). Following 24 h of SI, we previously observed a decrease in latency for rats to fall asleep in a multiple sleep latency test, an increase in basal forebrain adenosine, and an increase in non-REM delta power during recovery sleep, all proposed to be measures of sleepiness (McCoy et al. 2007; McKenna et al. 2007; Tartar et al. 2006). Attentional and cognitive deficits following SI have been documented both in animals (Christie et al. 2008; Cordova et al. 2006; McCoy et al. 2007; Tartar et al. 2006; Ward et al. 2009) and in humans (Dinges et al., 1997). In particular, in a hippocampal-dependent spatial learning task (water maze) our laboratory previously reported that 24 h of SI prior to acquisition in Sprague-Dawley rats (SD) resulted in deficits of both acquisition and retention (Tartar et al., 2006), whereas in Fischer/Brown Norway rats (FBN) only deficits in retention were observed (i.e., memory for the hidden location of the platform; Ward et al., 2009).

The chronic intermittent hypoxia (IH) exposure of OSA is thought to impair cognition via apoptosis in cortex and CA1 region of the hippocampus (Banasiak and Haddad 1998; Gozal et al. 2001; Kawasaki et al. 1990). Spatial memory impairments, as measured by water maze performance in rodents, have been observed following exposure to as little as 3 days of IH exposure (Goldbart et al. 2003a, 2003b; Gozal et al. 2001; Gozal et al. 2003a, 2003b; Gozal et al. 2002; Kheirandish et al. 2005a, 2005b). In most of these studies, performance deficits were also observed following 7 or 14 days of IH treatment (12 h/day). Alterations in rates of neuronal apoptosis (Gozal et al. 2003b), as well as formation of reactive species (i.e., oxidized free radicals), may underlie some of the deleterious consequences associated with chronic intermittent hypoxia (Lavie 2003; Veasey et al. 2004). It is not known, however, if short periods of IH (e.g., 24 h) can impair cognition, possibly via apoptosis or other unidentified mechanisms. This question is important to address because acute exposure to low levels of oxygen at high altitudes can produce cognitive impairments via neuronal apoptosis in hippocampus, cortex and striatum (Maiti et al., 2008). Acute high altitude exposure can produce high altitude periodic breathing of sleep (West, 2004), and acute altitude sickness, is characterized, in part,

by cognitive deficits and sleep disruption (Hornbein, 2001; Shukitt-Hale et al., 1998). Hence, this study was also designed to test the prediction that a 24 h period of SI will impair spatial memory greater than will acute (24 h) exposure to IH..

The aim of this study was to assess cognitive function in the rat, employing a one-day version of the water maze task. By comparing the effects of 24 h of exposure to either SI or IH, each treatment's relative contribution to acute cognitive impairment was evaluated. After our preliminary findings indicated that 24 h of IH did not impair acquisition or retention, we added an experimental group designed to replicate the previously published finding that as little as 3 d of IH exposure (10 h/day) would impair place acquisition (Goldbart et al., 2003b). While we acknowledge that the time course of the acute exposures to SI and IH protocols (24 h) is not comparable to that associated with chronic sleep apnea in humans, these experiments allowed an evaluation of the acute effects of SI versus IH on cognition, permitting each of these symptoms to be studied in isolation. Also, experiments were designed to allow evaluation of SI or IH exposure either before or after acquisition, in order to distinguish the specific effects of each manipulation on acquisition of spatial information (learning) versus retention (consolidation and memory of spatial information).

2. Results

For the 24 h SI and 24 h IH protocols, we utilized a version of the water maze task that allowed rats to be fully trained in 1 day (Frick et al., 2000b): Fischer/Brown Norway (FBN) rats underwent three blocks of four trials separated by 30-min rest periods. This protocol made it possible to conduct manipulations that cannot be given on multiple days. The 3 day exposure to IH (10h/day) utilized a 2 day water maze protocol in order to replicate the published procedures of Goldbart et al. (2003b) as follows: four blocks of learning trials (4 trials per block) divided over two days (see Methods for further details). SI or IH were presented either prior to, or after, water maze acquisition training, as illustrated in Figure 1.

2.1 Experimental Sleep Fragmentation

For SI exposure, the treadmill ran for 30 s (30s on), followed by no treadmill movement for 90s (90s off), continuously producing 30 interruptions of sleep per hour for 24 hours. To control for motor involvement and the effects of stress associated with exercise, a separate group of exercise control (EC) rats were exposed to a schedule on the treadmills of 10 min on and 30 min off. Thus, the EC condition produced the same total amount treadmill movement per 24h, but allowed for longer periods of consolidated sleep in order to control for the possible confounds of exercise/stress caused by the treadmill procedure. A third group, cage control (CC), was also analyzed, where animals lived in the treadmill cage but were not exposed to treadmill movement.

Twenty-four hours of SI before acquisition had no significant effect on FBN rats' performance during acquisition ($F(2,28) = 0.626$, $p > 0.05$) or on retention of the hidden platform location as measured in a probe trial 24h after acquisition training ($F(2,28) = 1.164$, $p > 0.05$; Figure 2A and 2B). No significant differences were noted in swimming velocity, indicating swimming ability was normal ($F(2,28) = 0.606$, $p > 0.05$; data not shown).

As seen in Figure 2D, 24h of SI after acquisition (training) significantly impaired retention on the probe trial. This difference in performance on the probe trial was significant as measured by the change in percent distance spent in the correct quadrant ($F(2,28) = 5.851$, $p = 0.008$). Post-hoc tests revealed that SI after acquisition significantly reduced the percent distance and percent time that animals spent in the correct quadrant, relative to both the cage controls (CC; $p = 0.015$) and the exercise controls (EC post; $p = 0.014$). While exercise controls spent a mean ($\pm$ SEM) of 45.83 ($\pm$ 3.29) % of their time in the correct quadrant, animals exposed to 24 h SI

after acquisition spent only 24.21 (± 1.84)% of their time in the correct quadrant, a value indicating chance levels of performance (25%). No significant differences were noted in acquisition trials ($F(2,28) = 0.602$, $p > 0.05$). Additionally, no significant differences were observed in swim speed velocity ($F(2,28) = 0.414$, $p > 0.05$; data not shown).

2.2 Intermittent Hypoxia

Our intermittent hypoxia (IH) regimen is a modified version of the protocol originally developed by Gozal and associates (Gozal et al., 2001b). Essentially, atmospheric O₂ were cycled between 6 and 19% within a custom designed airflow restricted chamber, modeling the episodes of hypoxemia associated with sleep apnea without necessarily affecting sleep (Gozal et al., 2001a).

As illustrated in Figure 3, 24 h of IH exposure either before, or after, acquisition training had no significant effect on performance during acquisition ($F(2,26) = 0.113$, $p > 0.05$, top panel) or retention as assessed by the probe trial ($F(2,26) = 1.142$, $p > 0.05$, bottom panel). As one might expect, there were no pre-existing differences in rate of acquisition between the treatment group that received 24 h of IH after acquisition (IH post) and room air controls (AC).

In a separate experiment (Figure 4), a group of rats were given 3 d of IH and tested using a different water maze protocol (see 4.4. Water Maze Procedure for details). Following 3 d of IH exposure (10 h/day), prior to acquisition, rats showed a significant impairment in place acquisition as measured by the distance swam to find the hidden platform ($F(3,42) = 3.259$, $p < 0.05$). Specifically, rats exposed to 3 days of IH were impaired on the first two acquisition training sessions on the first day of testing. There was no significant difference in the percent of swim distance rats spent in the target quadrant during the probe trial ($F(1,14) = 0.491$, $p > 0.05$). There were no significant differences between groups in swim velocity ($F(1,14) = 0.142$, $p > 0.05$; data not shown).

3. Discussion

The present experiments were undertaken to compare the effects of acute (24 h) experimental sleep fragmentation (sleep interruption; SI) and acute (24 h) intermittent hypoxia (IH) on spatial learning and memory in Fischer/Brown Norway (FBN) rats. SI and IH were presented either before, or after, water maze acquisition training in order to begin to dissociate the effects of these manipulations on learning versus memory. Interestingly, 24 h of SI placed prior to acquisition training had no effect, whereas SI placed after the acquisition training significantly impaired memory. This is the first evidence in an animal model that experimental sleep fragmentation after acquisition impairs spatial memory. The ability of 24 h of SI placed after learning to impair memory is consistent with evidence from both human (Maquet et al., 2000b) and animal (Louie and Wilson 2001; Skaggs and McNaughton 1996) studies illustrating the importance of uninterrupted sleep after initial learning for the consolidation of labile memories. In contrast to SI, 24 h of IH exposure, either prior to, or following acquisition, had no significant effect on either learning or memory. Finally, to verify a previous report (Goldbart et al. 2003b) a group of rats was exposed to 3 days of IH (10 h/day) prior to acquisition training and this treatment significantly slowed learning the platform location. The present data is consistent with the recent finding of Perry et al. (2008) which indicate that rats following paradoxical sleep deprivation were impaired in forming an avoidance task memory while IH exposed rats showed no effect.

Recent human studies demonstrate the importance of individual differences in sleep physiology (Tucker et al., 2007) that can also predict the cognitive and performance deficits associated with sleep disruption (Bliese et al. 2006; Tucker et al. 2007). Different rat strains also vary in susceptibility to the deleterious effects of sleep disruption, which resembles the individual

variation observed in humans. Substantial baseline differences in water maze performance are known to exist between the two rat strains we have studied, with FBN rats outperforming Sprague-Dawley (SD) rats (Harker and Whishaw, 2002). 6 h of total sleep deprivation (Guan et al., 2004) and 24 h of SI (Tartar et al., 2006) placed prior to water maze acquisition training impaired learning and memory in SD rats in previous studies, but did not impair the performance of FBN rats herein. The findings suggest that the water maze performance of the SD rat strain is more susceptible to SI than is the performance of the FBN rat strain. This discussion also suggests that the superior ability of FBN rats in the water maze may render this strain less susceptible to the impairments induced by SI, and, in turn, the SD rat strain is predicted to be even more susceptible to SI if the 24 h of SI is placed after acquisition training in the water maze, a prediction that remains to be tested. Finally, the fact that the retention of platform location in FBN was impaired when SI followed acquisition but not if SI preceded acquisition suggests that memory consolidation is more readily disrupted by 24 h SI than is acquisition/learning. However, the difficulty of the water maze protocol used is also an important variable as we recently found that 24 h of SI before learning trials disrupted 24 h retention in FBN rats when using a more demanding version of the water maze task with 8 massed trials (Ward et al., 2009).

The SI-induced performance deficits in the water maze may be mediated by a disturbance in hippocampal dependent memory consolidation. Several laboratories have reported that neuronal circuits in the hippocampus involved in mediating acquisition are reactivated during sleep episodes that follow the initial learning, in both rats (Louie and Wilson 2001; Skaggs and McNaughton 1996) and humans (Maquet et al., 2000a). Moreover, the amount of hippocampal re-activation during slow wave sleep in humans is directly proportional to next-day improvement on a navigational task (Peigneux et al., 2004). An induced form of synaptic plasticity, hippocampal long term potentiation (LTP), is a commonly accepted model of hippocampal memory processes (Shapiro and Eichenbaum, 1999). Tartar et al. (Tartar et al., 2006) reported in the rat that as little as 24 h of SI exposure was sufficient not only to impair spatial memory in the water maze, but also to eliminate hippocampal LTP. Finally, while training on hippocampal dependent tasks enhances survival of newborn neurons, sleep restriction has been found to reverse the increase in hippocampal neurogenesis induced by training (Guzman-Marín et al. 2003, 2007; Hairston et al. 2005).

The relative importance of NREM versus REM sleep in memory consolidation is a key question that is not directly addressed in the present study. The effect of our treadmill induced SI procedure on sleep architecture has been reported (McCoy et al. 2007; McKenna et al. 2007; Tartar et al. 2006) and both similarities and differences are observed between the two rat strains. 24 h of SI reduced the average duration of NREM sleep episodes in both strains, though the difference was greater in FBN (McCoy et al., 2007d) rats (from a baseline of 182 s to 56 s) than in SD (McKenna et al. 2007; Tartar et al. 2006) rats (from a baseline of 109 s to 63 s). In terms of the proportion of total time spent in NREM sleep, total (24 h) NREMS time declined significantly in FBN rats from 50% during baseline to 33% during SI (McCoy et al., 2007c). SD rats spent 41% of total time in NREM sleep during baseline compared to 37% during SI (McKenna et al. 2007; Tartar et al. 2006). While the direction of change was similar for both strains, the difference in total time spent in NREM sleep in SD rats was not statistically significant. In FBN rats (McCoy et al., 2007b), REM sleep was absent during SI (7% during baseline and 0% during SI). In SD rats (McKenna et al. 2007; Tartar et al. 2006), total time spent in REM sleep was significantly reduced from 13% during baseline to 3% during SI. Time spent awake increased proportionately in both strains (from 43% to 67% in FBN rats (McCoy et al., 2007a); from 46% to 60% during SI in SD rats (McKenna et al. 2007; Tartar et al. 2006)). Thus, the 24 h SI protocol had a more disruptive effect on sleep in general in FBN rats than in SD rats. Despite the greater disruption of sleep time and sleep architecture caused by

24h SI in FBN rats, the cognitive performance of the FBN rat strain is less impaired by sleep disruption compared to SD rats.

SD rats exposed to SI exhibit sleepiness, as indicated by a decrease of sleep onset latency in a multiple sleep latency test (McKenna et al. 2007), an increase of NREM delta power (McKenna et al. 2007; Tartar et al. 2006), and an elevated adenosine level within the basal forebrain (McKenna et al. 2007). When administered prior to acquisition, both total sleep deprivation (Guan et al. 2004) and selective REM sleep deprivation (Beaulieu and Godbout, 2000) have been shown to impair spatial memory in the water maze in SD rats. Impaired retention in the water maze has also been reported in SD rats that have been selectively deprived of REM sleep for several hours after acquisition (McDermott et al. 2003; Smith and Rose 1996; Smith and Rose 1997). It remains to be determined whether the memory impairment observed in FBN rats in the present study was due primarily to the elimination of REM sleep or to the reduction of NREM sleep time, or to both.

In contrast to the cognitive impairment associated with SI, neither spatial acquisition nor retention were altered by 24 h of IH, regardless if IH occurred prior to, or after, acquisition. Deficits in water maze performance have been documented following chronic exposure to IH, lasting 3-14 days (Goldbart et al. 2003a, 2003b; Gozal et al. 2001; Gozal et al. 2003a, 2003b; Gozal et al. 2002; Kheirandish et al. 2005a, 2005b). In one study, 3 days of IH exposure (40 cycles/h, during 12 h of the light phase) lead to impaired acquisition in SD rats (Goldbart et al. 2003b). In the present study, 3 days of IH exposure (30 cycles/h; 10 h/day) in FBN rats also impaired acquisition, although the impairment was only observed on the first two blocks of training sessions, administered on the first day of acquisition training. Learning deficits were not observed on the third and fourth blocks of acquisition trials which occurred on the second day of acquisition training, again indicating that the effects of 3 days IH exposure are milder in FBN rats compared to SD rats (Goldbart et al., 2003b). Unlike the study of Goldbart et al. (2003b), we did not observe a performance impairment on the probe trial following 3 days of IH and acquisition training, a minor discrepancy considering that Goldbart et al. (2003b) reported this probe trial effect in only one of several water maze experiments (Goldbart et al. 2003a, 2003b; Gozal et al. 2001; Gozal et al. 2003a, 2003b; Gozal et al. 2002; Kheirandish et al. 2005a, 2005b). More robust behavioral deficits have been reported with longer (7 or 14 days) exposures to IH (Goldbart et al. 2003a, 2003b). It could be that 3 days of IH is close to the minimum amount of IH that will produce detectable spatial memory impairments. On the other hand, behavioral deficits are not perfectly correlated with time course since 30 days of IH treatment does not result in water maze deficits (Goldbart et al., 2003b, Gozal et al., 2003a). Another factor that can explain differences in probe trial performance is the extent of learning during acquisition trials. In Goldbart et al (2003b), there was a significant difference in rats' performance in the final blocks of acquisition training, indicating that IH exposed rats had not learned the task as well as control rats; therefore it should not be a surprise that a recall test confirmed this. In the present experiment, performance was similar between groups of rats by the end of the task and likewise, there were no differences in later recall of the platform location.

In addition to behavioral deficits, a 3 day IH exposure has been found to diminish population spike-LTP in the hippocampal slice preparation (Payne et al., 2004). Moreover, behavioral deficits on this task have been temporally correlated with both rates of apoptosis, and decreases of phosphorylation of cyclic response element binding protein (CREB) in the hippocampus, an indicator of diminished plasticity (Goldbart et al. 2003b). Factors such as oxidative stress (Row et al. 2003; Veasey et al. 2004; Xu et al. 2004) and activity of inducible nitric oxide synthase (Li et al. 2004; Zhan et al. 2005) occur after as little as 1 to 3 days of IH exposure, whereas decreased function of Apolipoprotein E (Kheirandish et al. 2005b; O'Hara 2005) occurred after 14 days of IH exposure. Thus, multiple days of IH exposure are likely to be

necessary to impair spatial memory function through the disruption of hippocampal plasticity (Payne et al., 2004), through alternations in NMDA receptors (Gozal et al., 2001), and/or other proteins in the hippocampus (Goldbart et al., 2003a, Goldbart et al., 2003b). Additionally, increases in inflammatory response (Li et al., 2003, Li et al., 2004) and oxidative stress (Row et al., 2004) could play important roles. Other neurobiological effects of long term exposure to IH that are likely to negatively influence cognition include the loss of noradrenergic neurons of the locus coeruleus, dopaminergic neurons of the periaqueductal gray (Zhu et al., 2007), and damage to the cholinergic basal forebrain system (Row et al., 2007). Interestingly, hypoxia-induced deficits in water maze performance were prevented by pretreatment with an adenosine A1 receptor antagonist, suggesting that pharmacological treatment can compensate for some of the cognitive effects of long term IH exposure (Sun et al. 2002).

Previous research has shown that IH can disrupt sleep architecture during the first day of IH exposure in rats, and for longer periods in mice. However, unlike the SI condition, any sleep disturbance produced by the 24 h IH condition in the present study was not sufficient to alter water maze performance. Gozal et al. (2001) found that chronic IH in rats reduced NREM and REM sleep only during the first day of IH exposure, with sleep amounts returning to normal on days 2 to 14 (Gozal et al., 2001), findings that we have confirmed in unpublished observations. These findings indicate that it is highly unlikely that the water maze performance impairments observed in the 3 days of IH group are caused by sleep disturbances. In contrast to rats, the sleep of mice is more readily disrupted by chronic IH exposure with Polotsky et al. (2006) describing a decrease in NREM sleep during the first day and a significant decrease in REM sleep for up to five days (they attributed these sleep changes to the rapid changing of air and associated noise causing sleep fragmentation). Similarly, Veasey et al. (2004) found an increase in NREM sleep and no change in REM sleep during or immediately following 14 days of IH in mice. Despite the fact that 24 h of IH exposure has been shown to alter sleep in rats, any sleep disruption in the 24 h IH condition did not influence water maze performance in the present study.

In conclusion, 24 h of SI after acquisition training robustly impaired spatial memory while SI before acquisition had no significant effect on acquisition or retention of the hidden platform location in FBN rats. This suggests that spatial memory consolidation is more susceptible to sleep disruption than is spatial learning. In contrast, 24 h of IH, irrespective of whether it occurred prior to or after the initial learning, had no significant effect on performance in the water maze, indicating that acute IH does not produce cognitive impairments such as those seen in acute altitude sickness. On the other hand, 3 days of IH (10 h/day) before acquisition was sufficient to impair performance during acquisition indicating that longer durations of IH exposure are needed to produce impairments in spatial learning and memory.

4. Experimental Procedures

4.1. Subjects

Ninety young adult (60-80 day) male Fischer/Brown Norway (FBN) rats (Harlan Sprague-Dawley, Indianapolis, IN) were housed under constant temperature ($23 \pm 1^\circ \text{C}$) and a 12:12-h light-dark cycle (lights on at 0800 h). Food and water were available *ad libitum*. All procedures were conducted in accordance with the Policies on the Use of Animals and Humans in Neuroscience Research (Society for Neuroscience, 1995) and the Institutional Animal Care and Use Committee (IACUC) of the Boston VA Healthcare System.

4.2. Experimental Design

Treatment conditions consisted of independent groups ($n = 8-12$ rats/group) of subjects. Separate experiments evaluated the effects of 24 h of sleep interruption (or equivalent amounts

of exercise) either prior to or immediately following acquisition of the water maze task. Similarly, separate experiments tested the effects of 24 h of intermittent hypoxia (or room air) either prior to or immediately following acquisition. Figure 1 represents the timeline of the experimental protocols. A final experiment tested the effects of 3 days (10 h/day) of intermittent hypoxia.

4.3. Apparatus

The dimensions of the water maze were 2 m in width and 0.6 m in depth, containing opaque (nontoxic paint) water held at room temperature ($24 \pm 1^\circ \text{C}$). A tablespoon of bleach was added daily as an antibacterial agent. The pool was in the center of a 2.7×4.5 m room containing distal cues (i.e., posters with distinctive patterns) on each wall. Illumination was held constant in the room.

4.4. Water Maze Procedure

Each rat underwent three blocks of four trials separated by a 30-min period. This version of the water maze task allows rats to be fully trained in approximately 3 h (Frick et al., 2000a), which is necessary for manipulations that cannot be given on multiple days, such as 24-h sleep fragmentation. Rats were tested in the water maze during the last three hours of the 12-h lights-on period. On each trial, rats were placed in the water maze facing the wall in one of three quadrants that did not contain the hidden platform. The starting position was in a semi-random order so that no start point was repeated and no point was used more than four times. The location of the hidden platform remained constant. If the animal did not find the hidden platform within 60 s, the rat was guided to the platform by the experimenter and allowed to remain on the platform for approximately 10 s before being placed in a holding cage for an additional 60 s. A video tracking system (EzVideo Multi Track System, AccuScan, Columbus, OH) was utilized to record rodent behavior in the water maze. Twenty-four hours after the last training trial, a probe trial was conducted to test for retention of (i.e., memory for) the location of the hidden platform. For the probe trial, the platform was removed and each rat had a 30-s free swim in the maze. Following the probe trial, rats complete a visible platform version of the water maze, in which a flag was placed on the platform and rats were allowed four 60-s trials to find the location of the platform. This was a control for swimming ability, motivation, and to some extent, visual acuity. Between trial blocks, rats were group housed in a dry cage.

The water maze protocol used for the 3 day (10 h/day) exposure to IH was modified in order to replicate the experiment conducted by Goldbart et al. (2003b). The water maze testing followed 3 days of IH and rats were not exposed to IH during the 3 days of water maze testing. The same apparatus and handling procedures as those described above were utilized. Instead of a single day of training, four blocks of learning trials were divided over two days. Each day, rats received two blocks of training sessions separated by 4 h. Each block of training sessions consisted of 4 trials with an inter-trial interval of 2 min. On the third day, a probe trial was given following the protocol described above.

4.5. Sleep Interruption

The SI parameters were designed to model the sleep fragmentation of sleep apnea. Custom treadmills ($l \times w \times h = 50 \text{ cm} \times 16.5 \text{ cm} \times 30.5 \text{ cm}$) were designed to allow rats free access to food and water while inducing locomotor activity according to a programmed schedule. The treadmill methods were modified from Guzman-Marin et al. (Guzman-Marin et al., 2003). Rats were habituated to the treadmill for 2 days prior to experimentation. The treadmills for the sleep interrupted (SI) group were scheduled to be on for 30s and off for 90s continuously for 24 hours, creating 30 sleep interruptions per hour. Food and water was available *ad libitum* in the treadmill. A horizontal belt was automatically programmed to move at a rate of .02 m/s. We have previously determined that the sudden movement of the treadmill interrupts the rats'

sleep and produces sleep fragmentation, whereas rats are able to sleep during the 90 s of undisturbed time (McCoy et al. 2007; McKenna et al. 2007; Tartar et al. 2006). To control for motor involvement and the effects of stress associated with exercise, a separate group of exercise control (EC) rats were on a schedule of 10 min on and 30 min off. Thus, the EC condition produced the same total amount of time on the treadmill and exercise/stress per 24 h as those animals in the SI condition. However, the longer duration of treadmill operation (for the EC group) allowed for extended periods of undisturbed sleep. The EC procedure has been shown to produce a very small decrease in total sleep time (<10%) and no change in REM sleep time (McCoy et al. 2007; McKenna et al. 2007; Tartar et al. 2006). A third group of cage controls (CC) spent the same amount of time on the treadmills as SI and EC, except the treadmill was now turned off. SI rats were observed during the 30 min inter-trial intervals in order to prevent sleep with gentle handling and sensory stimulation; however, the SI rats engaged primarily in grooming and social interaction during this period.

4.6. Intermittent Hypoxia

All animals were first habituated to the custom designed cages ($l \times w \times h = 35.5 \text{ cm} \times 22.8 \text{ cm} \times 20.3 \text{ cm}$) with room air being continuously infused into the chamber for 48 h prior to the IH exposure. For IH exposure, the custom designed cages restricted airflow and were designed to allow internal O₂ levels to be cycled. Infused gas cycled inside the cage every 120 s with room air (21% O₂) to nitrogen rich air (6% O₂). O₂ levels were measured via an oximeter. Pilot data (not shown) has shown that rats are exposed to environmental oxygen on a schedule that produces hypoxia with a similar frequency observed in typical human with sleep apnea (30 hypoxic episodes/h) and producing hypoxemia that mimics the blood O₂ de-saturation typical of sleep apnea (70 to 75% O₂ de-saturation relative to normal blood O₂). Animals were exposed to the IH protocol for one continuous 24 h period starting at 1700 hours (i.e., 3 h prior to the start of the lights off cycle) using a sinusoidal exposure of 60 s of reduced environmental O₂. This exposure produces at least 20 s of inspired air levels below 10%, followed by 60 s of normal air, which produces at least 20 s of inspired air levels above 18% O₂. To produce the pattern of inspired O₂ levels in our specific cages, 8 L/min of N₂ for 120 s was followed by 11 L/min for normal air for 60 s. During the 60 s periods of normoxia, pilot data indicated that blood oxygen saturation always returned to normal. Rats had free access to food and water in the cages. Air control rats lived in a similar cage with room air continuously infused into the chamber for equivalent time periods.

A separate experiment was conducted to replicate the findings of Goldbart et al. (2003b). In this experiment, rats were given 3 consecutive days of IH exposure for 10 h each day beginning at lights on. All other aspects of the protocol, including habituation and flow parameters, were the same as those described above.

4.7. Data Analysis

The main dependent variable collected in all acquisition trials were path distance to find the hidden platform in the water maze. In probe trial data, the main dependent variable was percent swim distance spent in the quadrant of the pool that formerly contained the hidden platform. All data is expressed as mean ($\pm$ SEM). Water maze acquisition data were analyzed by a mixed model ANOVA with acquisition trials as the repeated measure. Simple effects were analyzed utilizing a 95% confidence interval. Probe trail data were analyzed by ANOVA followed by a Tukey post hoc test. All data analysis was conducted utilizing SPSS (v 13.0) with an alpha level of 0.05.

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References

- Banasiak KJ, Haddad GG. Hypoxia-induced apoptosis: effect of hypoxic severity and role of p53 in neuronal cell death. *Brain Res* 1998;797:295–304. [PubMed: 9666152]
- Beaulieu I, Godbout R. Spatial learning on the Morris Water Maze Test after a short-term paradoxical sleep deprivation in the rat. *Brain Cogn* 2000;43:27–31. [PubMed: 10857657]
- Bedard MA, Montplaisir J, Richer F, Rouleau I, Malo J. Obstructive sleep apnea syndrome: pathogenesis of neuropsychological deficits. *J. Clin. Exp. Neuropsychol* 1991;13:950–964. [PubMed: 1779033]
- Bliese PD, Wesensten NJ, Balkin TJ. Age and individual variability in performance during sleep restriction. *J Sleep Res* 2006;15:376–385. [PubMed: 17118094]
- Christie MA, Bolortuya Y, Chen LC, McKenna JT, McCarley RW, Strecker RE. Microdialysis elevation of adenosine in the basal forebrain produces vigilance impairments in the rat psychomotor vigilance task. *Sleep* 2008;31:1393–1398. [PubMed: 18853936]
- Cordova CA, Said BO, McCarley RW, Baxter MG, Chiba AA, Strecker RE. Sleep deprivation in rats produces attentional impairments on a 5-choice serial reaction time task. *Sleep* 2006;29:69–76. [PubMed: 16453983]
- Dinges DF, Pack F, Williams K, Gillen KA, Powell JW, Ott GE, Aptowicz C, Pack AI. Cumulative sleepiness, mood disturbance, and psychomotor vigilance performance decrements during a week of sleep restricted to 4-5 hours per night. *Sleep* 1997;20:267–277. [PubMed: 9231952]
- Frick KM, Stillner ET, Berger-Sweeney J. Mice are not little rats: species differences in a one-day water maze task. *Neuroreport* 2000;11:3461–3465. [PubMed: 11095500]
- Goldbart A, Cheng ZJ, Brittan KR, Gozal D. Intermittent hypoxia induces time-dependent changes in the protein kinase B signaling pathway in the hippocampal CA1 region of the rat. *Neurobiol. Dis* 2003a;14:440–446. [PubMed: 14678760]
- Goldbart A, Row BW, Kheirandish L, Schurr A, Gozal E, Guo SZ, Payne RS, Cheng Z, Brittan KR, Gozal D. Intermittent hypoxic exposure during light phase induces changes in cAMP response element binding protein activity in the rat CA1 hippocampal region: water maze performance correlates. *Neuroscience* 2003b;122:585–590. [PubMed: 14622901]
- Gozal D, Daniel JM, Dohanich GP. Behavioral and anatomical correlates of chronic episodic hypoxia during sleep in the rat. *J. Neurosci* 2001;21:2442–2450. [PubMed: 11264318]
- Gozal D, Row BW, Gozal E, Kheirandish L, Neville JJ, Brittan KR, Sachleben LR Jr. Guo SZ. Temporal aspects of spatial task performance during intermittent hypoxia in the rat: evidence for neurogenesis. *Eur. J. Neurosci* 2003a;18:2335–2342. [PubMed: 14622195]
- Gozal D, Row BW, Kheirandish L, Liu R, Guo SZ, Qiang F, Brittan KR. Increased susceptibility to intermittent hypoxia in aging rats: changes in proteasomal activity, neuronal apoptosis and spatial function. *J. Neurochem* 2003b;86:1545–1552. [PubMed: 12950463]
- Gozal E, Gozal D, Pierce WM, Thongboonkerd V, Scherzer JA, Sachleben LR Jr. Brittan KR, Guo SZ, Cai J, Klein JB. Proteomic analysis of CA1 and CA3 regions of rat hippocampus and differential susceptibility to intermittent hypoxia. *J. Neurochem* 2002;83:331–345. [PubMed: 12423243]
- Guan Z, Peng X, Fang J. Sleep deprivation impairs spatial memory and decreases extracellular signal-regulated kinase phosphorylation in the hippocampus. *Brain Res* 2004;1018:38–47. [PubMed: 15262203]
- Guzman-Marin R, Bashir T, Suntsova N, Szymusiak R, McGinty D. Hippocampal neurogenesis is reduced by sleep fragmentation in the adult rat. *Neuroscience* 2007;148:325–333. [PubMed: 17630219]
- Guzman-Marin R, Suntsova N, Stewart DR, Gong H, Szymusiak R, McGinty D. Sleep deprivation reduces proliferation of cells in the dentate gyrus of the hippocampus in rats. *J. Physiol* 2003;549:563–571. [PubMed: 12679377]

- Hairston IS, Little MT, Scanlon MD, Barakat MT, Palmer TD, Sapolsky RM, Heller HC. Sleep restriction suppresses neurogenesis induced by hippocampus-dependent learning. *J. Neurophysiol* 2005;94:4224–4233. [PubMed: 16014798]
- Harker KT, Whishaw IQ. Place and matching-to-place spatial learning affected by rat inbreeding (Dark-Agouti, Fischer 344) and albinism (Wistar, Sprague-Dawley) but not domestication (wild rat vs. Long-Evans, Fischer-Norway). *Behav. Brain Res* 2002;134:467–477. [PubMed: 12191833]
- Hornbein TF. The high-altitude brain. *J Exp Biol Sep*;2001 204(Pt 18):3129–32. [PubMed: 11581326]
- Kawasaki K, Traynelis SF, Dingledine R. Different responses of CA1 and CA3 regions to hypoxia in rat hippocampal slice. *J. Neurophysiol* 1990;63:385–394. [PubMed: 2158521]
- Kheirandish L, Gozal D, Pequignot JM, Pequignot J, Row BW. Intermittent hypoxia during development induces long-term alterations in spatial working memory, monoamines, and dendritic branching in rat frontal cortex. *Pediatr. Res* 2005a;58:594–599. [PubMed: 16148079]
- Kheirandish L, Row BW, Li RC, Brittian KR, Gozal D. Apolipoprotein E-deficient mice exhibit increased vulnerability to intermittent hypoxia-induced spatial learning deficits. *Sleep* 2005b;28:1412–1417. [PubMed: 16335482]
- Lavie L. Obstructive sleep apnoea syndrome--an oxidative stress disorder. *Sleep Med. Rev* 2003;7:35–51. [PubMed: 12586529]
- Li RC, Row BW, Gozal E, Kheirandish L, Fan Q, Brittian KR, Guo SZ, Sachleben LR Jr, Gozal D. Cyclooxygenase 2 and intermittent hypoxia-induced spatial deficits in the rat. *Am J Respir Crit Care Med* 2003;15:469–475. [PubMed: 12773326]
- Li RC, Row BW, Kheirandish L, Brittian KR, Gozal E, Guo SZ, Sachleben LR Jr, Gozal D. Nitric oxide synthase and intermittent hypoxia-induced spatial learning deficits in the rat. *Neurobiol. Dis* 2004;17:44–53. [PubMed: 15350964]
- Louie K, Wilson MA. Temporally structured replay of awake hippocampal ensemble activity during rapid eye movement sleep. *Neuron* 2001;29:145–156. [PubMed: 11182087]
- Maiti P, Singh SB, Mallick B, Muthuraju S, Ilavazhagan G. High altitude memory impairment is due to neuronal apoptosis in hippocampus, cortex and striatum. *J Chem Neuroanat* 2008;36:227–238. [PubMed: 18692566]
- Maquet P, Laureys S, Peigneux P, Fuchs S, Petiau C, Phillips C, Aerts J, Del FG, Degueldre C, Meulemans T, Luxen A, Franck G, van der LM, Smith C, Cleeremans A. Experience-dependent changes in cerebral activation during human REM sleep. *Nat. Neurosci* 2000;3:831–836. [PubMed: 10903578]
- McCoy JG, Tartar JL, Bebis AC, Ward CP, McKenna JT, Baxter MG, McGaughy J, McCarley RW, Strecker RE. Experimental sleep fragmentation impairs attentional set-shifting in rats. *Sleep* 2007;30:52–60. [PubMed: 17310865]
- McDermott CM, LaHoste GJ, Chen C, Musto A, Bazan NG, Magee JC. Sleep deprivation causes behavioral, synaptic, and membrane excitability alterations in hippocampal neurons. *J. Neurosci* 2003;23:9687–9695. [PubMed: 14573548]
- McKenna JT, Tartar JL, Ward CP, Thakkar MM, Cordeira JW, McCarley RW, Strecker RE. Sleep fragmentation elevates behavioral, electrographic and neurochemical measures of sleepiness. *Neuroscience* 2007;146:1462–1473. [PubMed: 17442498]
- O'Hara R. The role of hypoxia in apolipoprotein E4-associated cognitive impairment: implications for neurodegeneration and sleep-disordered breathing. *Sleep* 2005;28:1355–1357. [PubMed: 16335323]
- Payne RS, Goldbart A, Gozal D, Schurr A. Effect of intermittent hypoxia on long-term potentiation in rat hippocampal slices. *Brain Res* 2004;17:195–199. [PubMed: 15542074]
- Peigneux P, Laureys S, Fuchs S, Collette F, Perrin F, Reggers J, Phillips C, Degueldre C, Del FG, Aerts J, Luxen A, Maquet P. Are spatial memories strengthened in the human hippocampus during slow wave sleep? *Neuron* 2004;44:535–545. [PubMed: 15504332]
- Perry JC, D'Almeida V, Lima MM, Godoi FR, Vital MA, Oliveira MG, Tufik S. Intermittent hypoxia and sleep restriction: motor, cognitive and neurochemical alterations in rats. *Behav Brain Res* 2008;3:373–380. [PubMed: 18329112]
- Polotsky VY, Rubin AE, Balbir A, Dean T, Smith PL, Schwartz AR, O'Donnell CP. Intermittent hypoxia causes REM sleep deficits and decreases EEG delta power in NREM sleep in the C57BL/6J mouse. *Sleep Med* 2006;7:7–16. [PubMed: 16309961]

- Roehrs T, Conway W, Wittig R, Zorick F, Sickelsteel J, Roth T. Sleep-wake complaints in patients with sleep-related respiratory disturbances. *Am. Rev. Respir. Dis* 1985;132:520–523. [PubMed: 4037527]
- Row BW. Intermittent hypoxia and cognitive function: implications from chronic animal models. *Adv Exp Med Biol* 2007;618:51–67. [PubMed: 18269188]
- Row BW, Liu R, Xu W, Kheirandish L, Gozal D. Intermittent hypoxia is associated with oxidative stress and spatial learning deficits in the rat. *Am. J. Respir. Crit Care Med* 2003;167:1548–1553. [PubMed: 12615622]
- Row BW, Kheirandish L, Cheng Y, Rowell PP, Gozal D. Impaired spatial working memory and altered choline acetyltransferase (CHAT) immunoreactivity and nicotinic receptor binding in rats exposed to intermittent hypoxia during sleep. *Behav. Brain Res* 2007;177:308–314.
- Row BW, Kheirandish L, Li RC, Guo SZ, Brittian KR, Hardy M, Bazan NG, Gozal D. Platelet-activating factor receptor-deficient mice are protected from experimental sleep apnea-induced learning deficits. *J Neurochem* 2004;89:189–196. [PubMed: 15030403]
- Shapiro ML, Eichenbaum H. Hippocampus as a memory map: synaptic plasticity and memory encoding by hippocampal neurons. *Hippocampus* 1999;9:365–384. [PubMed: 10495019]
- Shukitt-Hale B, Banderet LE, Lieberman HR. Elevation-dependent symptom, mood, and performance changes produced by exposure to hypobaric hypoxia. *Int. J. Aviat. Psychol* 1998;8:319–334. [PubMed: 11542275]
- Skaggs WE, McNaughton BL. Replay of neuronal firing sequences in rat hippocampus during sleep following spatial experience. *Science* 1996;271:1870–1873. [PubMed: 8596957]
- Smith C, Rose GM. Evidence for a paradoxical sleep window for place learning in the Morris water maze. *Physiol Behav* 1996;59:93–97. [PubMed: 8848497]
- Smith C, Rose GM. Posttraining paradoxical sleep in rats is increased after spatial learning in the Morris water maze. *Behav. Neurosci* 1997;111:1197–1204. [PubMed: 9438789]
- Sun MK, Xu H, Alkon DL. Pharmacological protection of synaptic function, spatial learning, and memory from transient hypoxia in rats. *J. Pharmacol. Exp. Ther* 2002;300:408–416. [PubMed: 11805198]
- Tartar JL, Ward CP, McKenna JT, Thakkar M, Arrigoni E, McCarley RW, Brown RE, Strecker RE. Hippocampal synaptic plasticity and spatial learning are impaired in a rat model of sleep fragmentation. *Eur. J. Neurosci* 2006;23:2739–2748. [PubMed: 16817877]
- Thomas RJ, Rosen BR, Stern CE, Weiss JW, Kwong KK. Functional imaging of working memory in obstructive sleep-disordered breathing. *J. Appl. Physiol* 2005;98:2226–2234. [PubMed: 15677733]
- Tucker AM, Dinges DF, Van Dongen HP. Trait interindividual differences in the sleep physiology of healthy young adults. *J Sleep Res* 2007;16:170–180. [PubMed: 17542947]
- Veasey SC, Davis CW, Fenik P, Zhan G, Hsu YJ, Pratico D, Gow A. Long-term intermittent hypoxia in mice: protracted hypersomnolence with oxidative injury to sleep-wake brain regions. *Sleep* 2004;27:194–201. [PubMed: 15124711]
- Verstraeten E. Neurocognitive effects of obstructive sleep apnea syndrome. *Curr Neurol Neurosci Rep* 2007;7:161–166. [PubMed: 17324368]
- Ward CP, McCarley RW, Strecker RE. Experimental sleep fragmentation impairs spatial reference but not working memory in Fischer/Brown Norway rats. *J Sleep Res* 2009;18:238–244. [PubMed: 19645967]
- West JB. The Physiologic Basis of High Altitude Disease. *Ann Intern Med* 2004;141:789–800. [PubMed: 15545679]
- Xu W, Chi L, Row BW, Xu R, Ke Y, Xu B, Luo C, Kheirandish L, Gozal D, Liu R. Increased oxidative stress is associated with chronic intermittent hypoxia-mediated brain cortical neuronal cell apoptosis in a mouse model of sleep apnea. *Neuroscience* 2004;126:313–323. [PubMed: 15207349]
- Zhan G, Fenik P, Pratico D, Veasey SC. Inducible nitric oxide synthase in long-term intermittent hypoxia: hypersomnolence and brain injury. *Am. J. Respir. Crit Care Med* 2005;171:1414–1420. [PubMed: 15750040]
- Zhu Y, Fenik P, Zhan G, Mazza E, Kelz M, Aston-Jones G, Veasey SC. Selective loss of catecholaminergic wake active neurons in a murine sleep apnea model. *J. Neurosci* 2007;27:10060–10071. [PubMed: 17855620]

Figure 1.

Illustration of the experimental design for exposure to SI or IH. 24 h of sleep interruption (SI) or 24 h intermittent hypoxia (IH) was placed either before (A) acquisition training in the water maze (WM) or after (B) acquisition training in the WM. Panel C illustrates the protocol for 3 days of IH (10 h/day) presented prior to WM training (2 blocks of 4 trials per day). In all experiments, the probe trial was used to assess the retention of spatial memory 24 h after the conclusion of acquisition training. Bottom bars represent time passage with black bars representing 12 h of lights off and white bars representing 12 h lights on. Top bars represent treatment with diagonal lines representing either SI/EC or IH exposure.

Figure 2. 24 h SI exposure prior to acquisition does not affect performance in the water maze whereas 24 h SI exposure after acquisition impairs later retention of the platform location
A. In the 3 blocks of acquisition trials (4 trials/block), there were no differences in the distance swam to the hidden platform among the following three groups of rats: Cage Controls; CC (n=12); Sleep Interruption; SI pre (n=9); Exercise Controls; EC (n=10). **B.** 24 h SI prior to acquisition also had no effect on retention, as measured by percent distance spent in the correct quadrant during a single probe trial run 24 h after acquisition training. **C.** Performance during acquisition did not differ across blocks of trials when 24 h SI occurred after acquisition (note: this acquisition training was run prior to SI or EC exposure, hence, all rats were “untreated”): Cage Controls; CC (n=12); Sleep Interruption; SI post- (n=9); Exercise Controls; EC (n=10). Note that the SI post group had not received SI exposure at the time of acquisition training. **D.** 24 h SI after acquisition robustly impaired retention on the probe trial run 24 h after acquisition training. Specifically, animals in the SI post group spent significantly less time and distance in the correct quadrant than either cage controls or exercise controls. * $p < 0.05$

Figure 3. 24 h IH exposure prior to, or after, acquisition does not alter acquisition or retention of platform location in the water maze

A. In the 3 blocks of acquisition trials, there were no significant differences in the distance swam to find the hidden platform among the following three conditions: Air Controls (AC; $n=9$) and Intermittent Hypoxia including the IH pre ($n=11$) and IH post ($n=9$) conditions. Note that the IH post group had not received IH exposure at the time of acquisition training. **B.** 24 h of IH exposure prior to, or after, acquisition training had no effect on retention of platform location, as measured by percent distance spent in the correct quadrant during a single probe trial run 24 h after acquisition training.

Figure 4. Three days (10 h/day) of IH exposure impairs acquisition of the water maze platform location

A. A three day exposure to IH (10 h/day), prior to water maze testing, significantly impaired performance during acquisition, as measured by the distance swam to find the hidden platform. Rats exposed to IH ($n = 8$) were impaired on the first two blocks of acquisition trials on day 1 when compared to Air Controls (AC; $n = 8$) (the vertical dashed line divides day 1 acquisition training from day 2). **B.** There were no significant differences in retention on the probe trial between the groups. Hence, three day exposure to IH (10 h/day) produced a mild impairment of place acquisition but did not alter retention for the hidden platform location. * $p < 0.05$