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Explicit memory, anxiety and depressive like behavior in mice exposed to chronic intermittent hypoxia, sleep fragmentation, or both during the daylight period

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ABSTRACT

Obstructive sleep apnea (OSA) is a chronic and highly prevalent condition characterized by chronic intermittent hypoxia (IH) and sleep fragmentation (SF), and can lead to a vast array of end-organ morbidities, particularly affecting cardiovascular, metabolic and neurobehavioral functioning. OSA can induce cognitive and behavioral and mood deficits.

Male C57Bl/6J 8-week-old mice were housed in custom-designed cages with a silent motorized mechanical sweeper traversing the cage floor at 2-min intervals (SF) during daylight for four weeks. Sleep control (SC) consisted of keeping sweeper immobile. IH consisted of cycling FiO_2 21% 90 seconds-6.3% 90s or room air (RA; FiO_2 21%) for sixteen weeks and combined SF-IH was conducted for nine weeks. Open field novel object recognition (NOR) testing, elevated-plus maze test (EPMT), and forced swimming test (FST) were performed.

SF induced cognitive NOR performance impairments in mice along with reduced anxiety behaviors while IH induced deficits in NOR performance, but increased anxiety behaviors. SF-IH induced impaired performance in NOR test of similar magnitude to IH or SF alone. Combined SF-IH exposures did not affect anxiety behaviors.

Thus, both SF an IH altered cognitive function while imposing opposite effects on anxiety behaviors. SF-IH did not magnify the detrimental effects of isolated SF or IH and canceled out the effects on anxiety. Based on these findings, the underlying pathophysiologic processes underlying IH and SF adverse effects on cognitive function appear to differ, while those affecting anxiety counteract each other.

1. Introduction

Obstructive sleep apnea (OSA) is a chronic and highly prevalent condition characterized by short recurrent interruptions in respiratory airflow during sleep. The breathing instability leads to episodes of intermittent hypoxia IH (Dempsey et al.

2010) associated with disrupted sleep due to recurrent arousals resulting in sleep fragmentation (SF)(Almendros et al., 2020; Badran et al., 2020; Dempsey et al., 2010). This relatively underdiagnosed syndrome affects around a billion people around the world (Benjafield et al., 2019) and constitutes a major public health issue. Patients with OSA may manifest excessive daytime sleepiness (He and Kapur, 2017) and have a wide array of end-organ morbidities, particularly affecting cardiovascular (Yeghiazarians et al., 2021) metabolic (Gileles-Hillel et al., 2016) and cognitive and behavioral functioning (Krysta et al., 2017; Lajoie et al., 2020).

Several human studies have documented significant changes in emotional and mood regulation, as well as in cognitive function in patients with OSA. Such deficits seem to extend beyond those associated with the underlying excessive sleepiness that is frequently the driving complaint, with deficits persisting in some patients even after therapeutic intervention (Sforza and Roche, 2012; Vanek et al., 2020). The magnitude of cognitive and behavioral impairments observed in OSA patients has been associated with both the severity of the disease (Thomas et al., 2005) and with the degree of hypoxemic burden (Bédard et al., 1991; Ward et al., 2009). Although both SF and IH likely contribute to the cognitive deficits seen in OSA, it is virtually impossible to extricate their relative contributions when evaluating OSA patients because SF and IH are concurrently present (Ward et al., 2009).

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Abbreviations:

EPMT	Elevated Plus Maze Test
FST	Forced Swimming Test
IH	Intermittent Hypoxia
NOR	Novel Object Recognition
OSA	Obstructive Sleep Apnea
RA	Room Air
SC	Sleep control
SF	Sleep Fragmentation
SF-IH	Sleep Fragmentation-Intermittent Hypoxia
SC-RA	Sleep control-Room air

To further evaluate this issue, murine models of OSA have been developed and have applied environmental episodic hypoxia to predictably mimic the oxyhemoglobin desaturations of OSA (Almendros et al., 2014; Chopra et al., 2016; Davis and O'Donnell, 2013; Farré et al., 2018; Song et al., 2015). Similarly, models of sleep disorders that mimic or replicate the SF patterns that characterize OSA have also been generated (Kaushal et al., 2012a; Nair et al., 2011a; Ramesh et al., 2012; Tartar et al., 2009; Toth and Bhargava, 2013; Ward et al., 2009; Yaoita et al., 2020). To date, the chronic combined effects of SF and IH on sleep, behaviors, cognition, or any other function have only been scarcely evaluated in rodents (Kaushal et al., 2012b; Wang et al., 2022).

A multitude of behavioral assays is currently available for assessing cognitive functions in mice. Among them, the Novel Object Recognition (NOR) test, which is based on the spontaneous behavior of mice to explore novelty in their surroundings, has emerged as a useful tool for the evaluation of explicit memory (Ennaceur, 2010; Gulinello et al., 2019; Leger et al., 2013). Anxiety and depressive like behaviors can in turn be evaluated with two complementary tests, namely the Elevated Plus Maze Test (EPMT) a test that is based on the innate, unconditioned fear that rodents display for open spaces and heights, with anxious animals spending more time in closed sectors (Walf and Frye, 2007), and the Forced Swimming Test (Can et al., 2012), which is based on the assumption that when placing an animal in a container filled with water, it will first make efforts to escape but eventually will become immobile, the latter reflecting a measure of behavioral despair (Yankelevitch-Yahav et al., 2015).

Here, we hypothesized that exposures to chronic IH and SF would promote reductions in NOR performance and increase both anxiety and depressive behaviors, and that their combination would further enhance such phenotypic changes.

2. Materials and methods

2.1. Animal model

All experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Missouri (IACUC #9586 and 9720).

Two hundred and ten male C57BL/6J mice (eight weeks old) were purchased from Jackson Laboratory (Bar Harbor, ME, USA). Animals were housed in a controlled environment with 12 h light–dark cycles (6 a.m.–6 p.m.) at constant temperature (24 ± 0.2 °C) with *ad libitum* access to food (normal chow) and water. All animals were allowed to recover and fully acclimate within the animal care facility for seven days upon arrival. Animals were then randomized into 6 different groups (SF and its sleep control (SC), IH and corresponding normoxic control (RA), and SF-IH and its control SC-RA). SF, IH or SF-IH conditions were applied 12 h per day during the light period (6 a.m.–6 p.m.).

2.1.1. Intermittent hypoxia (IH)

The IH exposure protocol used has been described in detail previously (Badran et al., 2020). Briefly, mice were exposed to cycles consisting of FiO_2 21% 90 s-6.2% 90s or room air (RA; FiO_2 21%) in commercially available environmental chambers (Oxycycler A44XO; BioSpherix, Redfield, NY, USA) during the light period for 16 weeks. The rest of the day (6p.m–6 a.m.), mice remained in normoxic conditions (21% FiO_2). These exposures recapitulate nadir oxyhemoglobin saturations in the range of 68–75%, which are the dominant correlate of moderate to severe OSA in humans (Farré et al., 2018).

2.1.2. Sleep fragmentation (SF)

The SF device used to induce sleep disruption has been previously described (Kaushal et al., 2012b; Nair et al., 2011a; Ramesh et al., 2012). Sleep arousals were induced by a mechanical horizontal bar sweeping just above the cage floor from one side to the other side of a standard mouse laboratory cage which was operated by a nearly silent motorized system. This automated device precludes the need for human intervention and minimizes any handling stress to the animals. To apply SF mimicking OSA, 2-min intervals between each sweep (i.e., 30 events/h) were applied during the light period (6 a.m.–6 p.m.) for a total period of 4 weeks. Mice were housed in custom-designed cages (SF) containing the SF apparatus (Model 80,391; Lafayette Instruments, Lafayette, IN). Sleep control (SC) conditions consisted of keeping the sweeper immobile.

2.1.3. SF-IH exposures

The SFIH exposure involved a combination of SF and IH protocols for a period of 9 weeks during which the SF cages resided inside the IH-environmental chambers. Animals were exposed at the same time to IH and SF during the light period.

2.2. Behavioral tests

Behavioral experiments were performed by operators who were blinded to the various treatments and conducted by three observers between 9 a.m. and 5 p.m. (Fig. 1). The behavioral test battery consisted of open field Novel Object Recognition (NOR) test, Elevated Plus Maze Test (EPMT) and Forced Swimming Test (FST).

For all animals, the tests were performed in the same order. Indeed, the FST must be the last test performed because this test induces stress for the animals, and can influence the results of the other experiments (Yankelevitch-Yahav et al., 2015). In addition, each test was performed during the same period of the day, because the period of the day can also have an impact on the behavior. Finally, the order of the animals was randomized.

Throughout the duration of the experiments, the experimental setups were cleaned with ethanol 70% to prevent odor cues. All mazes were purchased at Maze Engineers (Cambridge, MA, USA). EPMT and NOR were recorded from a vertical point of view with a video camera suspended above the experimental area. For the FST, a horizontal point of view was selected. All experiments were interfaced with a video tracking system (Noldus Ethovision XT16 Software, Leesburg, VA, USA).

2.2.1. Novel object recognition (NOR) test

The novel object recognition (NOR) trials were conducted to evaluate the preference for the novelty and acquisition of explicit memory as based on the tendency for mice to explore unfamiliar objects (Antunes and Biala, 2012; Leger et al., 2013). The task was performed in a set of four blue opaque open-field plastic chamber (40L x 40W × 30H cm per chamber). For each trial, mice were placed at the center of the open fields. On the first day of the test, two habituation trials were conducted for each animal, which consisted of a 10-min exploration period without any object in the open field arena.

On the second day, identically-shaped blocks of different colors (3L x 3W × 9.6H cm) were used as familiar objects, each mouse exposed to

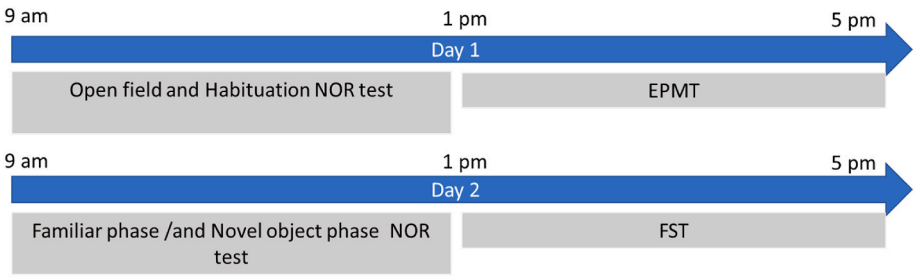

Fig. 1. Schematic representation of the behavioral experiment design. All experiment were conducted in the order shown.

two tower blocks in the same color. Objects were placed 5 cm from the side walls in the center of the arena. The mice were allowed to freely explore the objects for 5 min.

One hour later, one object was replaced by a novel object. We used a set of different novel objects. The mice were allowed to freely explore the objects for 5 min. Positive exploration by mouse was defined as touching the object with the nose.

The frequency and time spent exploring the objects were analyzed and quantified by the tracking software and was supervised by a blinded operator. To avoid object bias, the new object was placed randomly either on the left or on the right side of the arena, and the choice of familiar versus novel object was also randomly allocated.

The total expiration time for both objects was recorded. Results were reported as preference score using the following formula (Antunes and Biala, 2012; Hammond et al., 2004):

$$Preference\ score = \frac{\text{Time spent near to novel object}}{\text{Time spent near to all objects}} * 100$$

Mice that did not explore objects were removed from the experiment. Animals were considered to have a preference for novelty if their preference score was >50%.

2.2.2. Open fields

The open field is a test used for measuring locomotor activity and behavior in laboratory rodents (Lad et al., 2010; Seibenhener and Wooten, 2015). We used the habituation phase of the NOR test described above to measure the locomotor activity of the mice. The animals were allowed to explore the open field for 10 min, with locomotor activity monitored during the first 5 min. The distance moved and the velocity were analyzed and quantified by the tracking software while being supervised by a blinded operator.

2.2.3. Elevated Plus Maze Test (EPMT)

The EPMT was used to assess anxiety behaviors (Sweis et al., 2016; Walf and Frye, 2007). The apparatus consists of an elevated cross formed by two open arms and two closed arms (arm length 35 cm, wall height for closed arms 20 cm) made of blue Plexiglas radiating from a central platform to form a plus-sign. A 0.5 cm height wall was added to the open arms to prevent mice from falling, especially when they change their motion direction. The device is situated 56 cm above the floor. In our experiments, we considered as open arms, the open arms as well as the center zone. The open arms are considered by mice as a threatening area. Animals were placed in the central area facing one open arm and allowed to explore the maze for 5 min. The time spent in the open arms is commonly used as a measure of impulsivity, while the time spent on the closed arms is deemed to reflect anxiety-like behaviors in mice.

2.2.4. Forced swimming test (FST)

The task was performed in a set of 2 identical transparent cylindrical containers (diameter 19.5 cm, height 40 cm), with a depth of 15 cm of water at 25 ± 2 °C. The water was changed between each animal run. Walls were placed between the two cylinder and between the cylinder and the experimenter to avoid any interference. Mice were individually placed and forced to swim in the cylinder for a total duration of 6 min. The immobility time, defined as the absence of escape-oriented behaviors, was scored for a total period of 4 min (the last 4 min), as previously described (Nair et al., 2011a). Each mouse was deemed as being immobile when it ceased struggling or swimming, and instead remained floating motionless in the water, making only those movements necessary to keep its head above water. Not moving was defined as the duration of time when the velocity of mouse motion decreased below 2 cm/s (Crowley et al., 2004). At the end of the experiment, mice were dried and placed under red head lamp until their fur was completely dry.

2.3. Statistical analysis

Statistical analysis was performed using Prism 9.2 for windows (GraphPad Software, San Diego, Ca, USAwww.graphpad.com). Unpaired t-tests were used for all the test except NOR test which does not have a normal distribution, and therefore a Mann-Whitney test was used instead. The data were expressed as mean ± SD. A p-value < 0.05 was considered statistically significant.

3. Results

Exposure to either SF for four weeks, IH for sixteen weeks, or a combination of IH and SF for nine weeks, did not affect locomotion, either regarding the distance moved (Fig. 2 A to C) or the velocity (Fig. 2 D to F) (see Fig. 3A and 3B).

Exposures to four weeks of SF induced significant decreases in the NOR preference scores (38.98 ± 32.8%) when compared to SC (59.39 ± 30.5%, p = 0.006) (Fig. 3A). Exposures to IH for sixteen weeks also induced a significant decrease in the NOR test performance (45.02 ± 39.4%) compared to RA mice (70.30 ± 24.7%, p = 0.0035) (Fig. 3B). After nine weeks of SF-IH exposures, the NOR preference score was reduced (42.24 ± 28.3%) compared to SC-RA conditions (63.79 ± 30.9%, p = 0.031) (Fig. 3C).

Exposure to SF during four weeks induce an increase in the time spent in open arms during the EPMT tests revealed reduced anxiety behavior (34.77 ± 8.6%) in SF-exposed mice compared to SC mice (29.4 ± 8.8%, p = 0.0059) (Fig. 4A). In contrast, the time spent in open arms was decreased in mice after 16 weeks of IH (IH: 27.59 ± 8.9% vs RA: 32.05 ± 10.2%, p = 0.029) (Fig. 4B). No differences emerged when mice were exposed to both SF and IH for 9 weeks (Fig. 4C). Regarding the frequency in open arms, no differences were observed for the mice exposed to SF (Fig. 4D) and SF-IH (Fig. 4F). The frequency in open arms for mice exposed to IH (22.53 ± 7.4) was decreased compared to RA mice (26.06 ± 8.7, p = 0.0408). As anticipated, the time spent in the closed arms corresponds to the reciprocal of the time spent in the open arms. Exposure to SF for four weeks induced a decrease in the time spent in the closed arms (65.23 ± 8.6%) compared to SC mice (70.60.4 ± 8.8%, p = 0.0059) (Fig. 4G). In contrast, the time spent in open arms was increased in mice after 16 weeks of IH (IH: 72.41 ± 8.9% vs RA: 67.95 ± 10.2%, p = 0.029) (Fig. 4H) while no differences emerged when mice were exposed to both SF and IH for 9 weeks (Fig. 4I) (see also Fig. 5).

Exposures to SF for 4 weeks or to SF-IH for nine weeks induced no

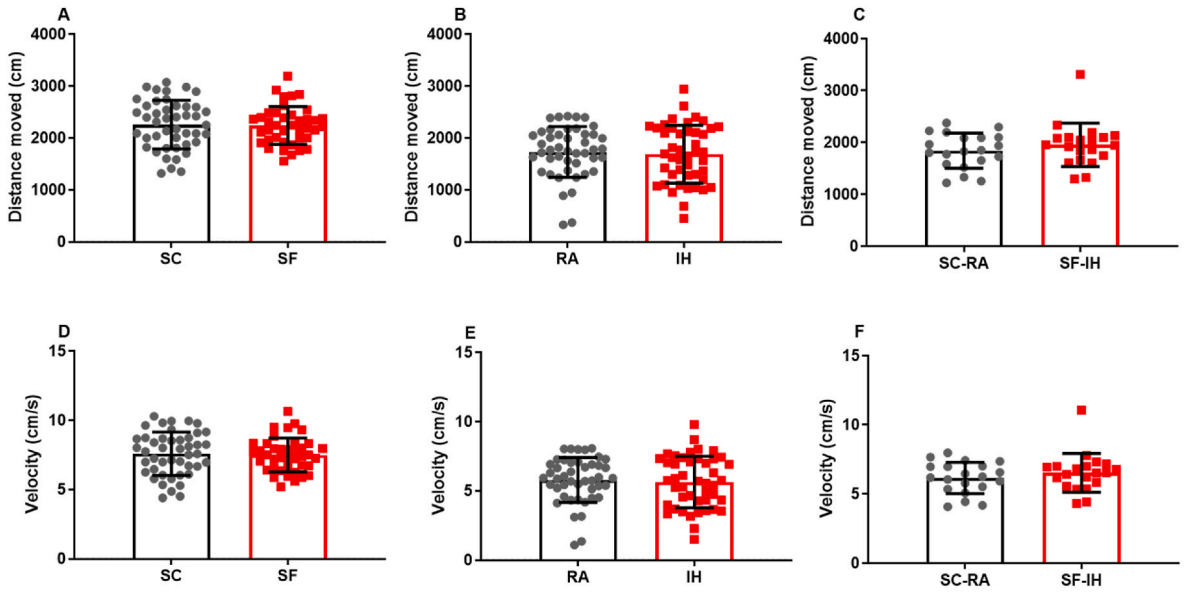

Fig. 2. Motor activity during open field in mice exposed to different models of OSA.

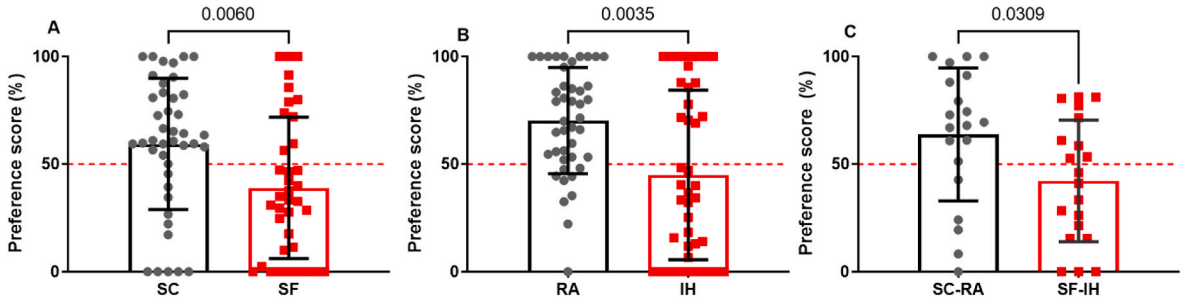

Fig. 3. Preference scores for the novel object recognition test in mice exposed to 4 weeks of SF (A), 16 weeks of IH (B) or 9 weeks of SF-IH (C) and plotted against their corresponding controls (SC, RA, SC-RA). Data are represented as mean ± SD, n = 39–43 for SF and IH and their controls and n = 20 for SF-IH and RA-IH control.

significant differences in immobility duration in the context of the FST when compared to SC or SC-RA mice, respectively (Fig. 5A &C). Sixteen weeks of IH induced significant decreases in the duration of immobility compared to RA-exposed mice (IH: 32.17 ± 10.4% vs RA: 39.12 ± 14.8%, p = 0.012) (Fig. 5B).

4. Discussion

This study shows similarities and differences in the cognitive and behavioral effects of chronic IH, SF, or their combination mimicking the cardinal perturbations recorded during sleep in patients with OSA. Considering the steady aging of the world population which is likely to further increase the prevalence of OSA, and the enhanced susceptibility to cognitive decline (Gozal et al., 2003; Shieu et al., 2022). It is of critical importance to better understand the consequences of such disease and its underlying mechanisms. Indeed, a wide range of cognitive deficits has been identified in OSA patients; these may range from altered attention and vigilance to deficits in memory and executive functions (Daulatzai, 2015). Our findings are congruent with the previous findings involving sleep manipulations or IH models of OSA in rodents. Indeed, cognitive impairments have emerged with sleep deprivation by exploring different learning and memory test paradigms, such as the Morris water maze (Nair et al., 2011a, 2011b; Tartar et al., 2006; Xie et al., 2020), contextual fear conditioning (Rossi et al., 2014; Sharma et al., 2021), inhibitory avoidance discrimination task (Bueno et al., 1994; Perry et al., 2008) and the NOR test (Djonlagic et al., 2012; Gozal et al., 2017; Kim et al., 2015; Palchykova et al., 2006). The latter NOR task has gained popularity in the investigation of memory alterations, since this test is based on the innate exploratory behavior of the rodent in the absence of externally applied rules or reinforcement (Antunes and Biala, 2012). In a previous study, we showed that IH induced time-dependent alteration in NOR performance in mice, and that such deficits became only partially reversible with incremental duration of the exposures (Gozal et al., 2017). Neither SF, IH or SF-IH exerted any effects on motor activity during the open fields test. These findings reinforce the differences found in the NOR tests, since such differences cannot be attributed to locomotion-related issues, and mice are therefore able to explore the novel object in same manner. In the literature there is a discrepancy about the effect of sleep disorders or IH on the motor activity of mice. Some studies have shown similar results to current findings (Andersen et al., 2005; Araujo et al., 2006; Meng et al., 2020; Onaolapo et al., 2016), while others found a modulation of locomotion (Baitharu et al., 2013; Grubac et al., 2019; Grubač et al., 2021; Yuan et al., 2015). The method of fragmentation used in those studies consisted of a treadmill forced locomotion, which can induce per se a reduction in motor activity, and as such the effect may not be attributable to SF. The effect of IH exposure on locomotor activity seems to correlate to the duration of the exposure (Baitharu et al., 2013).

Here, we expanded on the previous findings, and explored both IH and SF separately and their combination to explore whether there were any differences in the deleterious effects induced by the characteristic perturbations of OSA, namely IH and SF (Gozal et al., 2017; Kaushal et al., 2012a; Kim et al., 2015; Nair et al., 2011a; Palchykova et al., 2006; Ramesh et al., 2012; Shahveisi et al., 2020). Surprisingly and

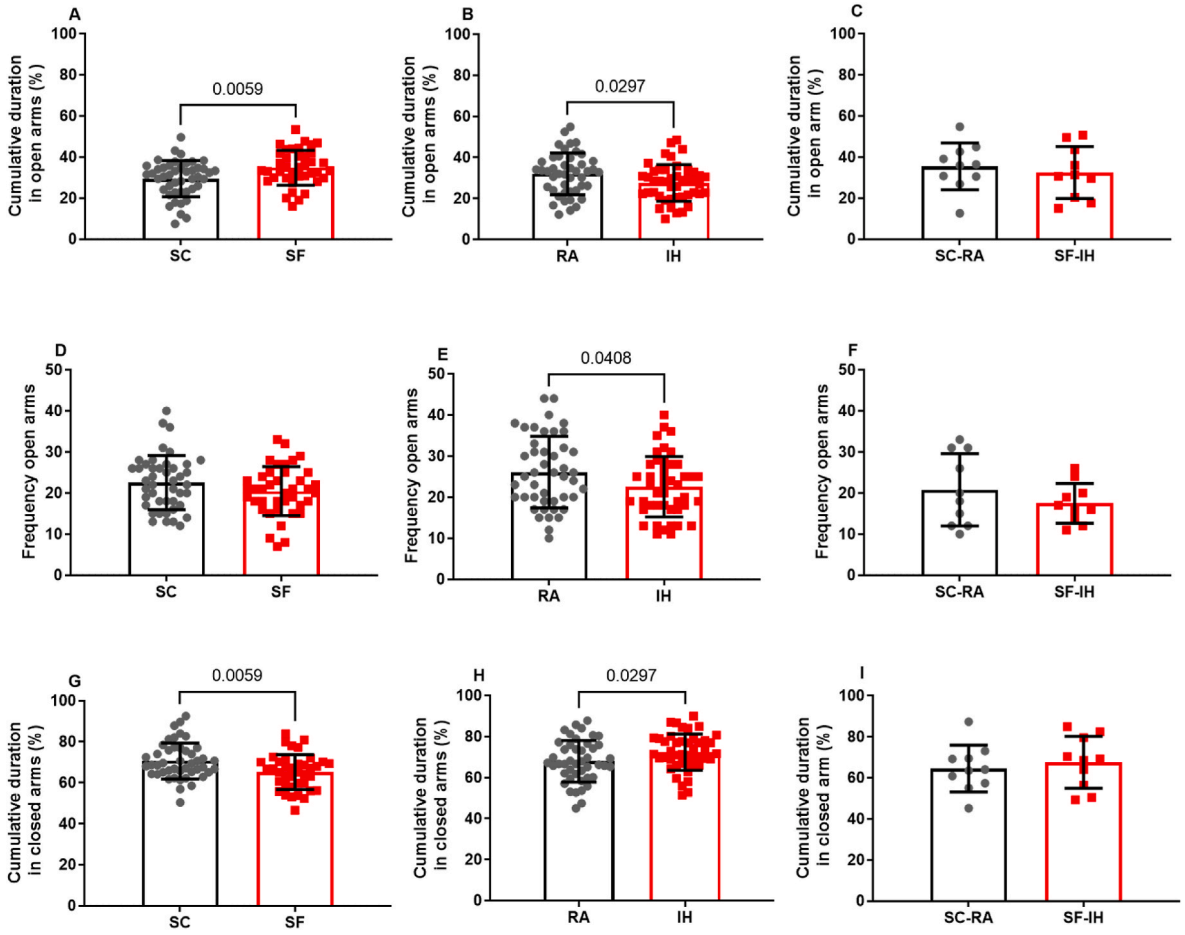

Fig. 4. Anxiety studies during Elevated Plus Maze test (EPMT) in different models of OSA The time spent in open arms is measured in mice exposed to 4 weeks of SF (A), 16 weeks of IH (B) or 9 weeks of SF-IH (C) the frequency in open arms is also measured in same animal: SF (D), IH (E), SF-IH (F). GHI corresponds respectively to the time spend in closed arms in each group: SF, IH, SF-IH. Each group is plotted against his corresponding controls (SC, RA, SC-RA)). Data are represented as mean ± SD, n = 39–45 for SF and IH and their controls and n = 10 for SF-IH and RA-IH control.

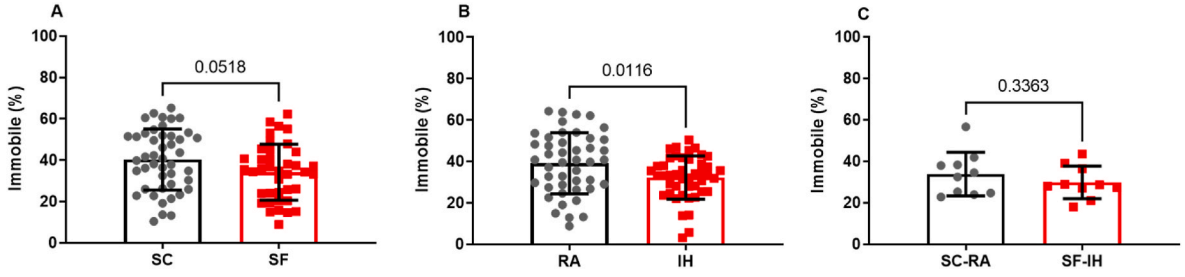

Fig. 5. Time spent immobile during the FST in mice exposed to 4 weeks of SF (A), 16 weeks of IH (B) or 9 weeks of SF-IH (C) and plotted against their corresponding controls (SC, RA, SC-RA). Data are represented as mean ± SD, n = 39–45 for SF and IH and their controls and n = 10 for SF-IH and RA-IH control.

contrary to our driving hypothesis, SF reduced anxiety behaviors while IH promoted them, with SF-IH resulting in the mutual cancelation of these effects. Similarly, no additive effect of IH and SF emerged in the context of the preference score in the NOR test when mice were exposed to SF-IH. These two observations may reflect divergent mechanistic pathways that underlie the neural substrate deficits that reflect the behavioral performances evaluated by these tests. As such, further exploration of such mechanisms and their interactions in the context of SF-IH seems a worthwhile endeavor.

Symptoms of anxiety and depression are both prevalent among patients with OSA (Akberzie et al.; Garbarino et al., 2020). Fearful mice would normally spend less time in the open arms of EPMT (Aduema et al., 2018), and in contrast, more time spent in the open arms could reflect either attenuation of open space contextual fear or increased inattention/impulsivity (Yaoita et al., 2020). The reduced duration in the time spent in open arm in IH-exposed mice would certainly seem to indicate an increase of anxiety (Abdel-Wahab and Abdel-Wahab, 2016; Fan et al., 2021; Nair et al., 2011b; Yuan et al., 2015). However, it seems that this effect is dependent on the time of exposure to IH, studies with a short exposure to IH (one week and less) have shown an anxiolytic effect (Carissimi et al., 2018; Perry et al., 2008; Zhu et al., 2010) while longer exposures (10 days and longer) induced anxiety in mice (Abdel-Wahab and Abdel-Wahab, 2016; Fan et al., 2021; Yuan et al., 2015). Also, a long IH exposure can induce excessive sleepiness and restricted sleep in

rodent models while such may not be the case during short exposures (Carissimi et al., 2018; Kaushal et al., 2012b; Veasey et al., 2004).

Several studies have reported increase in anxiety behaviors in mice exposed to a sleep disorder (Grubac et al., 2019; Nair et al., 2011a; Ramesh et al., 2012; Tai et al., 2020). As shown here, SF was not accompanied by the anticipated increases in anxiety and further resulted in decreased anxiety. However, there are few studies exploring the effects of SF, and these were all limited to short-term SF exposures (Patti et al., 2010; Tartar et al., 2009; Yaoita et al., 2020). Anxiety studies in rodents exposed to sleep deprivation have shown a decrease in anxiety similar to our results in SF exposed mice (Pires et al., 2016; Pokk and Väli, 2002; Suchecki et al., 2002). More studies with different exposure times, different periods of the day, and incorporation of tests that address these suppositions are needed. As such, future exploration of the temporal trajectories of the performance in each of the cognitive and behavioral tests used herein in the context of SF may uncover heretofore unknown patterns of response. We should remark that sleep deprivation induced by different methods (Patti et al., 2010; Pokk and Väli, 2002; Suchecki et al., 2002; Tai et al., 2020; Yaoita et al., 2020) can lead to different alterations in sleep architecture and basal anxiety levels of mice along with evidence of oxidative stress in the brain (Grubac et al., 2019; Grubač et al., 2021; Nair et al., 2011a; Tartar et al., 2009).

Intriguingly, the combination SF-IH yielded no differences in the EPMT when compared to control mice, suggesting that the effects of SF counterbalance the anxiogenic effect of IH. As a corollary to such findings, we do not assume that SF-IH does not induce anxiety states, but rather posit that these mice are likely vulnerable to exhibit both short episodes of high anxiety and bursts of inattention, impulsivity to the surrounding environment (Pires et al., 2015). Indeed, sleep disorders induce a complex set of changed behaviors composed of anxiety, impulsivity, manic-like behaviors in both mice and humans (Anderson and Platten, 2011; Killgore, 2010; Luca et al., 2013; Pires et al., 2016; Young et al., 2011). We should remark that even if the EPMT is the gold-standard method for evaluation of anxiety in rodents, this test (as well as other anxiety tests) is not able to identify whether longer time in open arms is the result of decreased anxiety or increased inattention/impulsivity (Pires et al., 2016).

The findings in the FST, a test that is based on the natural tendency of rodents to escape from water (Kraeuter et al., 2019) are suggestive that the higher level of anxiety induced by IH is associated with a decrease of depressive like behaviors as illustrated by reduced immobility behaviors during the FST (Nair et al., 2011b; Zhu et al., 2010). Contrary to our assumptions, no evidence of depressive like behaviors emerged following SF (Xie et al., 2020) and SF-IH.

Before we conclude, several limitations of the present study deserve mention. First, the IH method employed in our present study mimicked simulations of oxyhemoglobin desaturations during sleep in a mouse model, but did not reproduce all the physiological changes occurring in OSA patients, such as increased respiratory efforts and hypercapnic periods occurring during upper airway obstruction. It is possible that interactions between these components may either accentuate or mitigate the neurocognitive consequences induced by IH (Row et al., 2003). Carbon dioxide can be anxiogenic in rodents (Améndola and Weary, 2020). In this model, mice are exposed to IH during the light period for 12h, a period when mice are assumed to preferentially engage in sleep. However due to the polyphasic architecture of mouse sleep, mice are also exposed to IH during the daylight, which differs from OSA in humans. Moreover, we allocated only one single time point for each of the exposures, and since behavioral changes appear to exhibit duration dependencies additional time points would have been interesting to pursue (Baitharu et al., 2013; Carissimi et al., 2018). Expansion of such exposures to include several early and long-term durations would result in the need for a very large number of mice which would be extremely difficult to handle and assess concurrently. Third, we evaluated only male mice who were also relatively young. Consequently, the effects of such exposures in female mice and in aging or very young mice remains to be explored. Currently, to our knowledge, there is no suitable method to distinguish between reduced anxiety and impulsive behavior. Lastly, this is a descriptive study which did not investigate putative mechanisms mediating the behavioral and cognitive effects. Such mechanisms have been partially explored in previous studies evaluating a single type of exposure for shorter periods of time (Dayyat et al., 2012; Li et al., 2004; Nair et al., 2011b, 2013, 2018; Row et al., 2004). The mechanism involved in the neurophysiological changes associated with OSA are multiple and complex (Khuu et al., 2019; Yagishita et al., 2017). Numerous physiological processes, especially involving inflammatory pathways are involved in the neurobehavioral consequences of OSA (Lavie, 2015; Row, 2007; Zhou et al., 2016). Damage induced by IH or SF has been observed in different brain regions such as the hippocampus, the amygdala and the prefrontal cortex (Cai et al., 2010; Khuu et al., 2019; Xie et al., 2020). Also SF and IH can affect many cell types: neurons, astrocytes, microglia, and endothelial cells, either directly or as an indirect consequence of other cell dysfunction. (Gozal et al., 2003; Kaneshwaran et al., 2019; Khuu et al., 2019; Kumar et al., 2009).

In summary, mice exposed to two the major hallmarks of OSA, namely chronic IH and SF reveal divergent effects on anxiety and similar reductions in performance in the NOR test. However, combination of these two perturbations does not necessarily result in an additive or synergistic effect on memory and de facto cancels out the divergent effects of IH and Sf when imposed separately.

Contribution to the field statement (200 words max)

Obstructive sleep apnea (OSA) is a chronic and prevalent disorder characterized by chronic intermittent hypoxia (IH) and sleep fragmentation (SF). Human studies have documented significant changes in emotional regulation, mood, and cognitive functions in patients with OSA. Considering the ongoing aging of the world population, it is important to better understand the mechanisms and consequences of this disease. In patients with OSAS, it is impossible to evaluate the individual effects of IH and SF, although it is likely that both contribute to these morbidities. Nevertheless, the combined chronic effects of SF and IH on behavior and cognition have been rarely evaluated in rodents. In this study, we evaluated explicit memory, anxiety and depressive like behaviors in mice subjected to IH, SF and both combined. Our study shows that mice exposed IH or SF, the major hallmarks of OSA, show similar reductions in explicit memory but divergent anxiety responses. However, the combination of the two does not necessarily induce a synergistic or additive effect on memory and the divergent effects on anxiety are de facto eliminated.

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Distance moved and velocity are measured in mice exposed to 4 weeks of SF (A & D), 16 weeks of IH (B & E) or 9 weeks of SF-IH (C & F) and plotted against their corresponding controls (SC, RA, SC-RA). Data are represented as mean ± SD, n = 39–43 for SF and IH and their controls and n = 20 for SF-IH and RA-IH control.

CRediT authorship contribution statement

Clementine Puech: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft. **Mohammad Badran:** Conceptualization, Methodology, Formal analysis, Investigation. **Alexandra R. Runion:** Investigation. **Max B. Barrow:** Investigation. **Zhuanhong Qiao:** Investigation. **Abdelnaby Khalyfa:** Conceptualization, Methodology. **David Gozal:** Conceptualization, Methodology, Validation, Supervision, Writing – review & editing, Funding

acquisition.

Declaration of competing interest

The authors declare no conflict of interest.

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