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Short communication

One week of exposure to intermittent hypoxia impairs attentional set-shifting in rats

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ABSTRACT

Intermittent hypoxia (IH), a characteristic of sleep apnea, was modeled in Fischer Brown Norway rats (10 h/day for 7 days) followed by cognitive testing in an attentional set-shifting task. The ability to shift attention from one sensory modality (e.g., odor) to another (e.g., digging medium) was impaired, a finding that could not be attributed to deficits in attention, discrimination, learning, or motor performance. Instead, the deficit is likely to reflect impaired allocation of attentional resources of the working memory system.

Intermittent hypoxia (IH) and sleep fragmentation are primary characteristics of the sleep apnea syndromes. Adult patients with sleep apnea typically exhibit excessive daytime sleepiness, mood disturbances, and impaired cognition [1]. Evidence suggests that both IH [2] and sleep fragmentation [3] may contribute significantly to the observed cognitive deficits. Impaired memory has been observed in humans diagnosed with sleep apnea [4], as well as in laboratory rats exposed to IH to model sleep apnea [5]. While spatial memory deficits in rodents have been repeatedly observed following IH (see [2] for a review), fewer attempts have been made to model the deficits in executive functioning commonly seen in apneic patients.

In the present study, laboratory rats were tested in an attentional set-shifting task following 1 week (10 h/day for 7 days) of exposure to IH. The task used here was developed by Birrell and Brown [6] to provide a rodent-appropriate version of the human intradimensional/extradimensional (ID/ED) shifting task [7], a test similar in method and purpose to the more familiar Wisconsin Card Sorting Test (WCST) for humans. The rationale for employing the attentional set-shifting task is based on evidence of dysregulation of inputs to the prefrontal cortex (PFC) in humans diagnosed with sleep apnea [3,8,9].

The PFC is well known to play a central role in "executive functioning," a term which refers here to the parceling out of attentional resources in response to changing environmental demands by components of the working memory system, which holds information on-line for immediate use [10]. Studies employing positron emission tomography (PET) have shown that extradimensional shifting activates the dorsolateral PFC in humans [11]. Furthermore, humans who have sustained damage to the PFC exhibit impaired performance on problem solving tasks that require the subject to shift attention from one rule to another [7]. In rats, lesion studies have demonstrated a key role for the medial PFC in attentional set-shifting [6]. The present study evaluated the performance of rats on the ID/ED attentional set-shifting task following exposure to 1 week of IH (10 h/day for 7 consecutive days).

Fourteen adult male Fischer Brown Norway F1 rats (252–274 g; Harlan Laboratories Ltd) 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 infused into the chamber through two alternating air sources in order to mimic the experimental conditions of alternating flow rates of N2 and normal air described below. The habituation period to room air lasted for 48 h prior to the IH exposure. Rats were exposed to the IH protocol for 10 h/day (from 2 pm

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to midnight) for 7 consecutive days (lights on at 8 am, off at 8 pm). With this IH exposure schedule, the morning hours were available to habituate the rats to the ID/ED task apparatus (days 1 & 2) and to shape the rats to find food pellets buried in terra cotta pots (three to five shaping sessions occurred on days 1 to 6; see [12] for details). On the morning of day 7, the rats experienced exemplar training to acquaint the animals with the possible reward-response contingencies of the task [12], and then were returned to the IH cages for the final 10 h of IH exposure. The formal ID/ED task occurred on the morning of day 8.

The IH regimen is a modified version of the protocol originally developed by Gozal et al. [5] and previously described by us [13]. The custom-designed cages restricted airflow and were designed to allow the cage O_2 levels to be systematically varied. Infused gas cycled inside the cage from room air (21% O_2 at 11 L/min for 60 s) to nitrogen rich air (6% O_2 at 8 L/min for 60 s). Cage O_2 levels were measured via an oximeter. Thus, rats were exposed to environmental oxygen on a schedule that produced hypoxia with a similar frequency observed in typical human with sleep apnea (30 hypoxic episodes per hour); in rats this IH regimen does not significantly alter sleep after the first 24 h of exposure [5]. The resultant hypoxemia mimicked the blood O_2 de-saturation typical of sleep apnea (70 to 75% O_2 de-saturation relative to normal blood O_2 using methods previously described by McGuire et al. [14]). This exposure produced at least 20 s of inspired air levels below 10%, followed by 60 s of normal air, which produced at least 20 s of inspired air levels above 18% O_2. Rats had free access to water in the cages. Air control rats lived in a similar cage with identical flow rates of room air infusion (11 L/min for 60 s and 8 L/min for 60 s).

The attentional set-shifting task and food restriction procedure for rats has been described in detail previously [6,12]. Rats were ordered weighing 220 g, allowed food *al libitum* until they reached at least 250 g. Each rat had an initial body weight between 250 and 275 g at the start of the food restriction protocol. Rats were then given 12 g of dry food pellets per day until they reached 90% of their initial body weight (typically 9 to 14 days of food restriction). Rats were weighed daily and rats that went below their 90% threshold were given additional daily food. Food restriction continued during the 7 days of IH and rats were fed 12 g food per day prior to the daily IH exposure (~1 pm). Rats obtained additional food in the habituation, shaping, and ID/ED experimental procedures. Body weight relative to each rat's initial body weight on the last day of the study ranged from 89.5 to 93% (mean = 90.6%). Rats appeared healthy and groomed normally throughout the course of this experiment suggesting that stress or other non-specific effects of combining IH exposure and moderate food restriction were unlikely to produce the specific behavioral impairments observed.

Rats were trained to discriminate between two terra cotta pots to obtain a small food reward (~0.05–0.15 g bit of rat chow) for each correct response. The pots were placed within the goal box portion of the test apparatus, a large, clear plastic box (16 cm tall, 90 cm long, and 44 cm wide). An opaque, removable divider separated the goal box from the remainder of the apparatus, which functioned as the starting point for each rat. Testing in the set-shift apparatus was conducted during the lights on phase. The task involved two pairs of stimuli to be discriminated. Each discrimination was represented by the pair of pots (Table 1). One pot was deemed the "correct" choice, based on either digging medium (e.g., different color paper, beads, etc. that filled the pots) or odor (i.e., scented oils applied to the rim of the pots). See McCoy et al. [12] for a list of the specific digging media and odors that were used. To prevent rats from using olfactory cues to signal the correct pot, a small amount of powdered food was placed in the incorrect pot.

Following habituation and shaping (see [12] for details), animals were tested in the following series of discrimination tests: simple discrimination, compound discrimination, reversal 1, intradimensional shift, reversal 2, extradimensional shift, reversal 3. For each discrimination test, animals were required to reach a criterion of six consecutive correct responses (referred to as "trials-to-criterion") before moving on to the next discrimination. A correct response was determined by direct observation of digging in the pot containing the reinforcement. Prior digging in the unrewarded pot constituted an incorrect response and ended the trial. In the simple discrimination, one of the two dimensions (e.g., odor in Table 1) were held constant while the other was varied (i.e., brown versus white paper) with one of the two (e.g., brown) being reinforced. In the complex discrimination, both dimensions were varied, but only one dimension was relevant and reinforced. Thus, rats learned to discriminate between stimuli within the relevant dimension (e.g., digging medium) while disregarding the other dimension (e.g., odor). For reversals, the previously unattended stimulus was now reinforced (e.g., white paper). For the intradimensional shift, animals were required to apply the previously learned rule regarding which stimulus dimension predicts reward to two novel stimuli (see Table 1). For the extradimensional shift, animals were required to shift their attention away from the previously reinforced stimulus dimension (i.e., digging medium) to the previously irrelevant dimension (i.e., a specific odor is now reinforced). The literature indicates that the extradimensional shift is the component that is most susceptible to disruption, with deficits on the ED shift observed following numerous manipulations, including brain lesions, sleep manipulations, pharmacological treatments, etc. (see [15] for review).

Behavioral performance on the ID/ED task was analysed using one-way analyses of variance (ANOVA) for independent groups (i.e., IH-exposed and room air controls). A separate one-way ANOVA was conducted for each of the seven discrimination tests of the attentional set-shifting task. All analyses were performed using SPSS (version 12) for Windows.

Following 7 consecutive days (10 h/day) of IH, a selective impairment was found in the extradimensional shift component of the ID/ED attentional set-shifting task ($F_{(1,12)} = 19.15$, $P = 0.001$). IH-exposed animals required an average of 17.9 (±1.3) trials to reach criterion, while room air controls reached criterion in only 10.3 (±0.9) trials. This equates to nearly a 58% increase in trials required to reach criterion when making an extradimensional shift (see Fig. 1). There were no impairments on any of the other phases of the ID/ED task.

The intermittent hypoxia (IH) paradigm employed in the present study was adapted from the protocol used by Gozal et al. [5] to model the episodic occurrence of hypoxia, which characterizes the sleep apnea syndromes. We have previously reported that 3 d of IH (10 h/day) impaired spatial learning of rats in the Morris water maze [13]. We now report additional impairments in attentional set-shifting following a longer duration of IH (7 days for 10 h/day). Since significant differences were not found on the intradimensional shift component, the observed impairment cannot be interpreted as a general deficit in the ability to solve new discrimination problems. A general deficit in attention, cognition or motor performance is also unlikely, as general deficits would have affected multiple components of the ID/ED task. The lack of an effect on reversal learning rules out the possibility that animals exposed to IH might merely be perseverating. Rather, 1 week of exposure to IH specifically impaired the ability of rats to shift attentional set from one sensory modality (e.g., odor) to another (e.g., digging medium). This selective finding suggests that IH impairs the allocation of attentional resources of the working memory system that are associated with the concept of executive function and the prefrontal cortex.

It is of interest that both 24 h of sleep fragmentation [12] and the 7 days of IH exposure used herein produce very similar and specific deficits on the ED shift component of the ID/ED task. The sever-