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# Ondansetron and fluoxetine reduce sleep apnea in mice lacking monoamine oxidase A

C. Real[a,*], I. Seif[a], J. Adrien[b,c], P. Escourrou[a]

[a] Univ Paris-Sud, EA 3544, Sérotonine et Neuropharmacologie, F-92296 Châtenay-Malabry cedex, France
[b] UPMC Univ Paris 06, UMR S677, F-75013 Paris, France
[c] INSERM U 975, CRicm-Hôpital Pitié Salpêtrière, F-75013 Paris, France

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ABSTRACT

Prospective clinical trials addressing the role of serotonin (5-HT) in sleep apnea have indicated that the 5-HT uptake inhibitor fluoxetine is beneficial to some patients with obstructive apnea, whereas the 5-$HT_3$ receptor antagonist ondansetron seems of little value despite its efficacy in rat and dog models of sleep apnea (central and obstructive). Here, we examined the effect of these drugs in transgenic mice lacking monoamine oxidase A (Tg8), which exhibit ~3-fold higher rates of central sleep apnea than their wild-type counterparts (C3H), linked to their enhanced 5-HT levels. Acute ondansetron (2 mg $kg^{-1}$, intraperitoneal), acute fluoxetine (16 mg $kg^{-1}$) and 13-day chronic fluoxetine (1 or 16 mg $kg^{-1}$) decreased by ~80% the total (spontaneous and post-sigh) apnea index in Tg8 mice during non-rapid eye movement sleep, with no statistically significant effect on apnea in C3H mice. Our study shows that both drugs reduce the frequency of apneic episodes attributable to increased monoamine levels in this model of MAOA deficiency, and suggests that both may be effective in some patients with central sleep apneas.



## 1. Introduction

The prevalence and morbid consequences of sleep apnea have stimulated investigations of this breathing disorder over the past decades, but there is no widely effective pharmacotherapy for this condition (Smith et al., 2006). The pathogenesis of both the *obstructive and central forms of* sleep *apnea* is diverse, with a significant overlap between the two forms (White, 2005). Some studies are focused on putative serotonin (5-HT) mechanisms and serotonergic therapy (Veasey, 2003). As of yet, no large-scale clinical trials for serotonin drugs against sleep apnea have been done because small trials in patients with obstructive sleep apnea have shown no clear benefit (Hanzel et al., 1991; Kraiczi et al., 1999; Stradling et al., 2003; Carley et al., 2007). Rat and dog models have yielded more promising results (Radulovacki et al., 1998; Veasey et al., 1999; Carley and Radulovacki, 1999; Carley et al., 2001; Veasey et al., 2001; Carley and Radulovacki, 2005). Mouse gene targeting offers a new approach to the identification of susceptibility factors to sleep-related apnea (Nakamura et al., 2007; Real et al., 2007) and allows investigation of the efficacy of serotonin agents in genetically defined models of central sleep apnea, as shown with the serotonin synthesis inhibitor parachlorophenylalanine in a mouse strain lacking monoamine oxidase type A (MAOA) (Real et al., 2007). Here, we extend this work by investigating the effects of ondansetron and fluoxetine on sleep apnea in this same strain.

Levels of extracellular 5-HT in the central nervous system appear to be lower during sleep than during wake (Portas et al., 1998; Jacobs and Fornal, 1999). Given that 5-HT can stimulate breathing (Hodges and Richerson, 2008), including through 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors on respiratory motoneurons (Fenik and Veasey, 2003), it has long been hypothesized that insufficient 5-HT activity during sleep might predispose to apnea (Hanzel et al., 1991). Along this line, fluoxetine and paroxetine, two serotonin selective reuptake inhibitors (SSRIs), were shown to reduce the rate of sleep apnea during NREMS in patients with obstructive sleep apnea, but with a wide variability in individual responses (Hanzel et al., 1991; Berry et al., 1999; Kraiczi et al., 1999). In a study in rats, a low-dose regimen of fluoxetine had no consistent effect on central sleep apnea (Carley and Radulovacki, 2005).

On the other hand, ondansetron, an inhibitor of peripheral 5-$HT_3$ receptors, had no effect on obstructive sleep apnea in a 10-patient study (0.15 mg $kg^{-1}$, per os) (Stradling et al., 2003) but was shown to reduce mixed (central and obstructive) sleep apnea in the English bulldog (2 mg $kg^{-1}$, per os) and central sleep apnea in the Sprague–Dawley rat (1 mg $kg^{-1}$, intraperitoneally) during rapid eye movement sleep (REMS) (Radulovacki et al., 1998; Veasey et al., 2001; Carley and Radulovacki, 2008). Interestingly, the effect of ondansetron on sleep apnea in this rat model appears to be poten-

* Corresponding author at: Sérotonine et Neuropharmacologie, EA 3544, Faculté de pharmacie, 5 rue Jean-Baptiste Clément, 92296 Châtenay-Malabry cedex, France. Tel.: +33 1 46 83 53 78; fax: +33 1 46 83 53 55.
*E-mail address:* caroline.prenat@gmail.com (C. Real).

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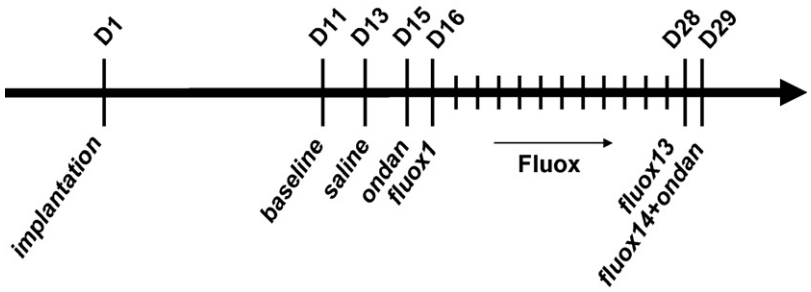


**Fig. 1.** Following electrode implantation (Day 1, D1, *implantation*), mice were allowed 10 days of recovery. Then, after baseline recording (D11, *baseline*), each animal was studied after saline (D13, *saline*) and ondansetron (D15, *ondan*) injections. Next, mice received daily injections of fluoxetine and were tested on the 1st (D16, *fluox1*) and 13th (D28, *fluox13*) days of treatment. On the 14th day, fluoxetine and ondansetron were co-injected (D29, *fluox14 + ondan*).

tiated by chronic fluoxetine (Carley and Radulovacki, 2005). This suggests that activation of the 5-HT system by fluoxetine combines with inhibition of peripheral 5-$HT_3$ receptors to reduce sleep apnea. However, other interpretations can be suggested because fluoxetine is known to have both serotonergic agonist and antagonist properties (Ni and Miledi, 1997; Eisensamer et al., 2003). Thus, the influence of fluoxetine on sleep breathing needs to be evaluated in genetic conditions of low, moderate, and high concentrations of 5-HT.

In the present study, we tested the effects of fluoxetine and ondansetron on sleep apnea in mice. We have recently shown that mice lacking monoamine oxidase A (MAOA), an important enzyme in the degradation of 5-HT and norepinephrine (NE) (Cases et al., 1995; Chen et al., 2004), are a model of central sleep apnea (Real et al., 2007), whereas the prevalence of sleep apnea in humans lacking MAOA has not been examined (Tuinier et al., 1995; Vossler et al., 1996). Adult MAOA knock-out (KO) mice with a C3H wild-type background display increased levels of 5-HT and NE (Cases et al., 1995) and increased rates of apnea in NREMS and REMS (Real et al., 2007). Their sleep apnea indices can be normalized by reducing 5-HT synthesis with parachlorophenylalanine (Real et al., 2007), suggesting that excess endogenous 5-HT facilitates sleep apnea, without excluding putative cofactors such as increased norepinephrine levels and perinatal effects of increased monoamine levels (Burnet et al., 2001). Here, we found that acute ondansetron, as well as chronic fluoxetine, induced a significant decrease in NREMS apnea in MAOA KO mice. Thus, in the presence of high endogenous baseline levels of serotonin, central sleep apnea in humans may respond favorably to fluoxetine or ondansetron.

## 2. Methods

### 2.1. Animals

Experiments were performed on mice of the C3H/HeOuJ strain (C3H, control mice) and its transgenic Tg(H2-IFN-β)8 strain (Tg8, MAOA-deficient mice). Tg8 mice were obtained following the injection of an interferon-beta cassette into a one-cell C3H embryo, leading to the insertional deletion of two essential exons in the MAOA locus (Cases et al., 1995). C3H and Tg8 mice were bred and raised under standard housing conditions in the transgenic animal facility of Paris-Sud University at Châtenay-Malabry (France). We used 2- to 3-month-old C3H and Tg8 males (20–25 g body weight). Because of the frequent fighting initiated by Tg8 males (Cases et al., 1995), both mutant and wild-type males were housed in individual cages (20 cm × 20 cm × 30 cm) from the age of 6 weeks, maintained in a ventilated cabinet with a 12:12-h light-dark cycle (lights on at 7:00 AM) and a temperature of 23 ± 1 °C, with food and water available ad libitum. All procedures involving animals and their care were conducted in conformity with institutional guidelines in compliance with national and international laws and policies (Council Directive # 87-848 of 19 October 1987, French Ministry of Agriculture and Forestry).

### 2.2. Surgical procedure

Mice were anesthetized with ketamine (100 mg $kg^{-1}$) plus xylazine (20 mg $kg^{-1}$) and enameled-insulated nichrome electrodes (150 μm in diameter) were implanted for polygraphic sleep monitoring, as previously described (Léna et al., 2004). Briefly, two electroencephalogram (EEG) electrodes were placed over the right cortex (2 mm lateral and 2 mm posterior to bregma) and over the cerebellum (2 mm posterior to lambda, at midline), two electrooculogram (EOG) electrodes were positioned subcutaneously on each side of the left eye, and two electromyogram (EMG) electrodes were inserted into the neck musculature. All electrodes were fixed to the skull with Super-Bond C&B (GACD, France) and the acrylic cement Dentalon Plus (GACD, France), and soldered to a connector also embedded in cement. The animals were allowed 10 days to recover from surgery before baseline recording (Fig. 1).

### 2.3. Sleep recording and scoring

Recordings were made during the light phase from 10:00 to 18:00. The night before experiments, mice were placed in the barometric chamber (see below) and connected to the recording cables. To allow freedom of movement to the animal, a slip ring was placed at the connection of the electrodes to the lines outside of the plethysmograph. The animal had food and water ad libitum and ambient temperature was maintained at 24 °C.

The EEG, EMG, and EOG signals were amplified and sampled by an EMBLA system (Medcare, Reykjavik, Iceland) and fed into a computer at a sampling frequency of 200 Hz for EEG and 100 Hz for neck EMG and EOG. Sleep recordings were scored in 5-s epochs by visual inspection of EEG, EOG, and neck EMG signals (Somnologica2 software, Medcare, Reykjavik, Iceland) using standard criteria to define wake, NREMS and REMS (Léna et al., 2004; Real et al., 2007). Vigilance state amounts were expressed as percent of the 8-h recording period.

### 2.4. Measurement of ventilation by whole body plethysmography

We used double-chamber whole-body plethysmography (Jacky, 1978). One chamber (500 ml, 10-cm internal diameter) was used as a barometric chamber where the mouse was placed, and reference pressure was measured in the other, each chamber being continuously flushed with room air at a rate of 700 ml $min^{-1}$ and outlet gas being monitored for $O_2$ and $CO_2$ to ensure that the outlet fractions of $O_2$ and $CO_2$ remain close to ambient air values (Elisa Duo, Engström, Denmark). The plethysmograph was placed in a circulating water bath set at 24 °C, in a ventilated room with a temperature