ORIGINAL ARTICLE

# Effects of Cannabinoid Agonists and Antagonists on Sleep and Breathing in Sprague-Dawley Rats

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**Study Objectives:** There are no pharmacological treatments for obstructive sleep apnea syndrome, but dronabinol showed promise in a small pilot study. In anesthetized rats, dronabinol attenuates reflex apnea via activation of cannabinoid (CB) receptors located on vagal afferents; an effect blocked by cannabinoid type 1 ($CB_1$) and/or type 2 ($CB_2$) receptor antagonists. Here, using a natural model of central sleep apnea, we examine the effects of dronabinol, alone and in combination with selective antagonists in conscious rats chronically instrumented to stage sleep and measure cessation of breathing.

**Methods:** Adult male Sprague-Dawley rats were anesthetized and implanted with bilateral stainless steel screws into the skull for electroencephalogram recording and bilateral wire electrodes into the nuchal muscles for electromyogram recording. Each animal was recorded by polysomnography on multiple occasions separated by at least 3 days. The study was a fully nested, repeated measures crossover design, such that each rat was recorded following each of 8 intraperitoneal injections: vehicle; vehicle and $CB_1$ antagonist (AM 251); vehicle and $CB_2$ antagonist (AM 630); vehicle and $CB_1$/$CB_2$ antagonist; dronabinol; dronabinol and $CB_1$ antagonist; dronabinol and $CB_2$ antagonist; and dronabinol and $CB_1$/$CB_2$ antagonist.

**Results:** Dronabinol decreased the percent time spent in rapid eye movement (REM) sleep. CB receptor antagonists did not reverse this effect. Dronabinol also decreased apneas during sleep, and this apnea suppression was reversed by $CB_1$ or $CB_1$/$CB_2$ receptor antagonism.

**Conclusions:** Dronabinol's effects on apneas were dependent on $CB_1$ receptor activation, while dronabinol's effects on REM sleep were CB receptor-independent.

**Keywords:** obstructive sleep apnea, dronabinol, cannabinoids, cannabinoid receptors, rat.

**Statement of Significance**

Poor adherence to continuous positive airway pressure, the gold standard treatment for obstructive sleep apnea (OSA), is an ongoing problem. New treatments for OSA are needed. There are no pharmacotherapies for OSA. This research shows the potential of dronabinol, a nonspecific cannabinoid receptor agonist, as a treatment for OSA.

## INTRODUCTION

Cannabinoids (CBs) impact on both sleep architecture[1–3] and respiratory pattern control,[4–6] but the mechanisms underlying these effects are not fully understood. Moreover, CB administration has been postulated as an innovative treatment for sleep-related breathing disorder,[7] which affects more than 25 million Americans.[8] The factors leading to apnea during non-rapid eye movement (NREM) versus rapid eye movement (REM) sleep are likely to be at least partially distinct.[9–13] Therefore, defining the mechanisms by which CBs influence both breathing pattern and sleep architecture will lend important insight into the potential utility of cannabimimetic pharmacotherapy for sleep-related breathing disorders.

Dronabinol, a synthetic nonselective CB type 1 ($CB_1$) and CB type 2 ($CB_2$) receptor agonist, has been shown to stabilize respiration during sleep in rats with spontaneous central apneas during sleep.[1] The clinical relevance of this observation is underscored by the fact that dronabinol was subsequently shown to ameliorate breathing disorder in patients with obstructive sleep apnea (OSA) syndrome;[14] a result that may reflect stabilization of respiratory pattern generation, increased activation of upper airway muscle activity, or other effects of dronabinol. In support of this view, recent experiments using a model of reflex apnea in anesthetized rats demonstrated that activation of CB receptors within the nodose ganglia suppressed 5-HT-induced apneas and increased respiratory phasic genioglossus muscle activity.[5] Furthermore, systemic antagonism of $CB_1$ or $CB_2$ receptors, individually or in combination, prevented dronabinol from suppressing 5-HT-induced apneas.[4]

Taken together, these findings suggest that dronabinol may act to reduce sleep-related breathing disorder by directly activating $CB_1$ and/or $CB_2$ receptors within the nodose ganglia. However, many CBs, including dronabinol, demonstrate significant activity within the CNS. Of particular relevance, CBs have the potential to suppress REM sleep, which has been suggested as a potential method of pharmacotherapy for sleep-related breathing disorders in its own right.[15] Further, CBs are known to exert nonreceptor mediated effects by allosterically modulating ionotropic receptors, including 5-$HT_{3A}$ receptors.[16–18] It remains unknown whether the suppression of both REM sleep and apneas by dronabinol in the conscious rat model reflects activation of $CB_1$ receptors, $CB_2$ receptors, or allosteric modulation of other receptors.

Here, we report that in chronically instrumented Sprague-Dawley rats, a natural animal model of spontaneous central sleep apnea,[19] dronabinol decreased REM sleep, an effect which was not blocked by CB receptor antagonists; and suppressed sleep apneas, an effect which was blocked $CB_1$ receptor antagonists.

## MATERIALS AND METHODS

### Animals

Adult male Sprague-Dawley rats ($n$ = 22; ~275 g) purchased from Harlan Laboratories (Indianapolis, IN) were initially housed in duplicate, maintained on a 12:12 hour light:dark cycle at 22 ± 0.5°C, and allowed ad libitum access to food and water. After surgery, rats were housed singly to prevent loss of

headsets. All animal procedures and protocols were approved by the Institutional Animal Care and Use Committee of the University of Illinois at Chicago.

### Surgical Procedures

Implantation of polygraphic headsets has been described before.[1,20] Rats were anesthetized (ketamine:xylazine 100:10 mg/kg; buprenorphine 0.1 mg/kg), stereotaxically immobilized, and implanted with electroencephalographic (EEG) screw electrodes bilaterally threaded into the frontal and parietal bones. Electromyographic (EMG) wire electrodes were implanted in the dorsal nuchal musculature and tunneled subcutaneously to the skull. EEG and EMG leads were soldered to a miniature plastic connector plug (i.e. headset) and affixed to the skull acrylic dental cement. Scalp wounds were closed with Vetbond Tissue Adhesive. Rats were allowed to recover for 7 days before beginning a week of acclimation to handling and to plethysmographic recording chambers.

### Polysomnography and Treatment Protocol

Polysomnography (PSG) procedures have been previously described.[20] Rats underwent nine 6-hour PSG recording, separated by at least 3 days. All recording sessions began at 10:00 and continued until 16:00. Each rat received an IP injection (1 mL/kg total volume) at 09:45. Rats were immediately placed inside a bias-flow-ventilated (2 L/min) whole-body plethysmograph (PLYUNIR/U, Buxco Electronics, Wilmington, DE), where respiratory airflow was detected by changes in pressure between the main chamber and an integrated reference chamber, as previously described.[9] A flexible cable was inserted through a narrow "chimney" into the main plethysmography chamber and attached to the rat's headset. Rats underwent a week of acclimation to handling and to plethysmographic recording chambers, including being connected to the flexible cable. After acclimation, rats were recorded for 6 hours for one occasion prior to the first experimental session to permit adaptation to the recording system, and to assess the quality of EEG and EMG signals. If signal quality was good, then the rats ($N$ = 8–10) underwent a repeated measures random order crossover design, such that each rat received each of 8 IP injections exactly one time in random order (i.e. any 8 of the IP injections could have been the first injection that a rat received): vehicle alone (DMSO; 1 mL); dronabinol (chemical name: (*6aR-trans*)-6a,7,8,10a-tetrahydro-6,6,9-trimethyl-3-pentyl-6*H*-dibenzo[*b,d*]pyran-1-ol) alone (10.0 mg/kg; Mylan Pharmaceuticals, Morgantown, WV); AM251 (chemical name: *N*-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide) alone (5.0 mg/kg, [$K_i$ = 7.49 nM], Tocris Bioscience, Bristol, UK); AM630 (chemical came: 6-Iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl](4-methoxyphenyl)methanone) alone (5.0 mg/kg, [$K_i$ = 31.2 nM], Tocris Bioscience); or AM 251/630 combination (5.0/5.0 mg/kg); or a combination injection (dronabinol and AM251 or AM630 or AM251/AM630). Respiratory signals were amplified, band-passed filtered (1 to 10 Hz; CyberAmp 380, Axon Instruments, Sunnyvale, CA), and digitized (250 samples/s; Bio-logic Sleepscan Premier, Natus, San Carlos, CA). EEG and EMG signals were amplified and band-passed filtered (0.5 to 100 Hz and 10 to 100 Hz, respectively) and digitized (250 samples/s; Bio-logic Sleepscan Premier). All data were stored to hard disk.

Visual scoring was conducted by a blinded and experienced technician. Sleep stages (wake, NREM, and REM) were scored for every 30-second epoch of the 6-hour recording. Wakefulness was characterized by high-frequency and low-amplitude (beta/alpha waves) EEG with high EMG tone. NREM sleep was characterized low-frequency and high-amplitude (delta waves) and low EMG tone, while REM sleep was characterized by high-frequency and high-amplitude (theta waves) EEG and an absence of EMG tone. Sleep stage percentages, defined as total time spent in a specific sleep stage (awake, NREM, or REM) divided by total time in the plethysmograph, and sleep efficiency, defined as total time spent in sleep (both NREM and REM) divided by total time spent in the plethysmograph, were also quantified. Sleep bouts were defined as NREM/REM sleep bounded by wakefulness, NREM bouts were defined as NREM sleep bounded by wakefulness and/or REM sleep, and REM bouts were defined as REM sleep bounded by wakefulness and/or NREM sleep. The average duration of those bouts were also quantified.

Apneas were scored as a cessation of breathing for at least 2 seconds, and were quantified as an apnea index (apneas/hour) and separately stratified for overall sleep and NREM sleep. Due to a small amount of time, or no time, spent in REM sleep, a REM apnea index was not calculated because there would be low estimation precision and many rats would have a "null" data point for REM apnea index. Since rats have an attached hyoid bone, all apneas observed were central rather than obstructive events. However, the brainstem neuronal circuity responsible for central and obstructive apneas overlaps,[21] and dronabinol has been shown to decrease obstructive apneas in humans.[14] Apneas were further subdivided into post-sigh (preceded by a breath at least 50% larger than the average of the preceding 5 breaths[22]) and spontaneous apneas (not preceded by an augmented breath), and shown as post-sigh and spontaneous apnea indices, respectively.[21,22] A sigh index was calculated for the entire time in the recording chambers (during awake and sleep). A "sigh" was defined as a breath that is 50% larger than the 5 preceding and 5 succeeding breaths. "Sniffing" was excluded from sigh analysis.

### Statistical Analysis

Data (mean ± SEM) were analyzed using IBM SPSS Statistics 22 (New York, NY) mixed model analysis using treatment (CB agonist, CB antagonist, and CB agonist/antagonist interaction) as a fixed effect and animal as a repeated measure, followed by post hoc multiple comparison tests with Sidak's correction if there were significant main effects or a significant interaction of main effects. Repeated covariance structure was chosen according to the best-fit Schwarz's Bayesian information criterion. Statistical significance was set at $p < .05$. Statistical trends were set at $.05 \leq p < .10$.

## RESULTS

A previous report from our lab showed decreases in apnea indices in rats receiving dronabinol dissolved in DMSO.[1] However, the exact mechanism of apnea suppression was not studied.

Rats ($N$ = 8–10) were injected with a CB receptor agonist (dronabinol; 10 mg/kg) or vehicle, and with $CB_1/CB_2$ receptor antagonists (AM251, AM630, or both; 5 mg/kg) or vehicle dissolved in DMSO (1 mL), and underwent PSG. Sleep efficiency is depicted in Figure 1 and time spent in wakefulness, NREM, or REM sleep is shown in Figure 2. Stratified apnea indices are presented in Figure 3.

There was a main effect of agonist treatment ($F_{1, 59.01}$ = 4.40, $p$ = .04) on sleep efficiency (Figure 1); dronabinol (56.93 ± 9.76%, $N$ = 34) decreased sleep efficiency compared to vehicle treated rats (62.16 ± 9.95%, $N$ = 39).

Time spent awake, or time spent in NREM or REM sleep was quantified (Figure 2). There was no effect of any treatment on time spent awake (Figure 2, left panel) or time spent in NREM sleep (Figure 2, middle panel). There was significant agonist/antagonist interaction ($F_{3, 48.90}$ = 4.23, $p$ = .01) observed for REM sleep. Post hoc analysis revealed significantly less ($p$ = .02) REM sleep in rats receiving dronabinol alone (1.26 ± 0.86%, $N$ = 10) compared to rats receiving vehicle only (3.67 ± 1.00, $N$ = 10), and rats receiving dronabinol and $CB_2$ antagonist had significantly ($p$ < .01) less REM sleep (0.85 ± 0.48%, $N$ = 8) compared to vehicle and $CB_2$ antagonist (4.29 ± 0.78%, $N$ = 10). Post hoc analysis also revealed that rats receiving vehicle and $CB_2$ antagonists (4.29 ± 0.78%, $N$ = 10) had more time spent in REM sleep than rats receiving vehicle and $CB_1$ antagonist (1.82 ± 0.78%, $N$ = 10) or vehicle and $CB_1/CB_2$ antagonist (1.90 ± 0.74%, $N$ = 9) treatment.

There were no differences in sleep bouts or NREM sleep bouts (data not shown). For REM bouts, there were trends for agonist effect ($F_{1, 31.86}$ = 3.54, $p$ = .07; data not shown) and antagonist effect ($F_{3, 49.07}$ = 2.54, $p$ = .07; data not shown). Post hoc analysis revealed that dronabinol (4.27 ± 1.17, $N$ = 34) tended to decrease REM bouts ($p$ = .07) compared to vehicle control (8.39 ± 1.25, $N$ = 39), and there was a trend of $CB_1$ antagonist (3.89 ± 1.44, $N$ = 18) to be decreased compared to $CB_2$ antagonist (8.50 ± 1.72, $N$ = 18; $p$ = .07). There were no differences in sleep bout duration or REM sleep bout duration (data not shown). There was a trend for an antagonist main effect ($F_{3, 15.58}$ = 2.66, $p$ = .09; data not shown) on NREM bout duration. However, post hoc analysis revealed no differences.

Dronabinol and/or CB antagonists showed significant differences in apnea indices measured by PSG. There was a significant agonist/antagonist interaction ($F_{3, 48.90}$ = 3.85, $p$ = .02) in the overall apnea index (Figure 3A). Post hoc tests showed that dronabinol alone (3.46 ± 0.73 events/hour, $N$ = 10) significantly decreased apneas ($p$ < .01) compared to vehicle control (9.00 ± 1.60 events/hour, $N$ = 10). There was a trend ($p$ = .07) for vehicle and $CB_1/CB_2$ antagonist (4.89 ± 1.09 events/hour, $N$ = 9) to decrease apneas compared to dronabinol and $CB_1/CB_2$ antagonist (8.63 ± 2.04 events/hour, $N$ = 8). Apnea suppression was significantly ($p$ = .03) reversed with dronabinol and $CB_1$ antagonist treatment (8.40 ± 2.13 events/hour, $N$ = 8) compared to dronabinol alone (3.46 ± 0.73 events/hour, $N$ = 10). There also was a trend ($p$ = .08) for apnea suppression to be reversed with dronabinol and $CB_1/CB_2$ antagonist treatment (8.63 ± 2.04 events/hour, $N$ = 8) compared to dronabinol alone (3.46 ± 0.73 events/hour, $N$ = 10). Though there was a difference in overall apnea index, there was no difference in apnea durations between any of the treatment groups (data not shown).

Apneas were divided into spontaneous and post-sigh, and then further into NREM spontaneous and NREM post-sigh apneas. There was a trend in the agonist/antagonist interaction ($F_{3, 49.91}$ = 2.32, $p$ = .09) on spontaneous apnea index (Figure 3B) and no effect of agonist or antagonist on NREM spontaneous apneas (data not shown). Post hoc analysis of spontaneous apneas revealed that dronabinol and $CB_1$ treatment (1.90 ± 0.58 events/hour, $N$ = 8) decreased ($p$ = .02) spontaneous apneas compared to vehicle and $CB_1$ treatment (4.67 ± 1.02 events/hour, $N$ = 10). There were significant agonist/antagonist interactions observed in post-sigh apneas ($F_{3, 56.06}$ = 4.91, $p$ < .01; Figure 3C) and NREM post-sigh apneas ($F_{3, 56.02}$ = 5.38, $p$ < .01; data not shown). Dronabinol alone (2.09 ± 0.50 events/hour,

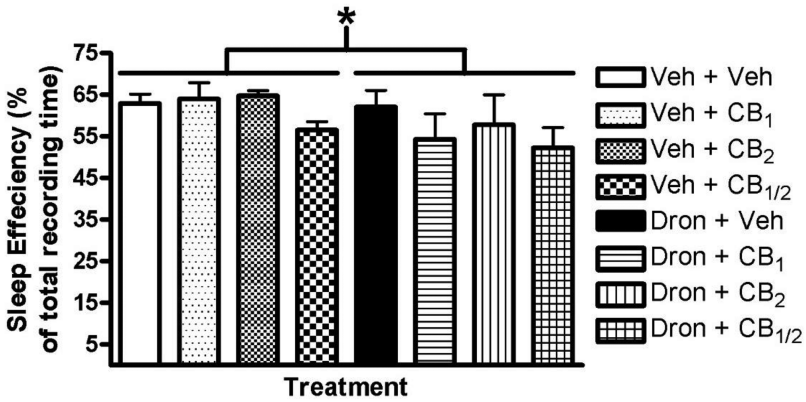


**Figure 1**—Sleep efficiency quantified as a percentage of time spent asleep from 6-hour recordings of conscious chronically instrumented rat experiments. Vehicle (DMSO) or dronabinol (10 mg/kg) was injected IP in combination with vehicle (solid bars) or $CB_1$ receptor (AM 251, 5 mg/kg) or $CB_2$ receptor (AM 630, 5 mg/kg) antagonist, or both (shaded bars). There was a significant agonist main effect; there was a decrease in sleep efficiency in the dronabinol treat rats. Data (mean ± SEM) were analyzed using mixed model analysis with repeated/fixed measures (CB agonist and CB antagonist) followed by post hoc multiple comparison tests with Sidak's correction if there were significant main effects or a significant interaction of main effects. *$p$ < .05.

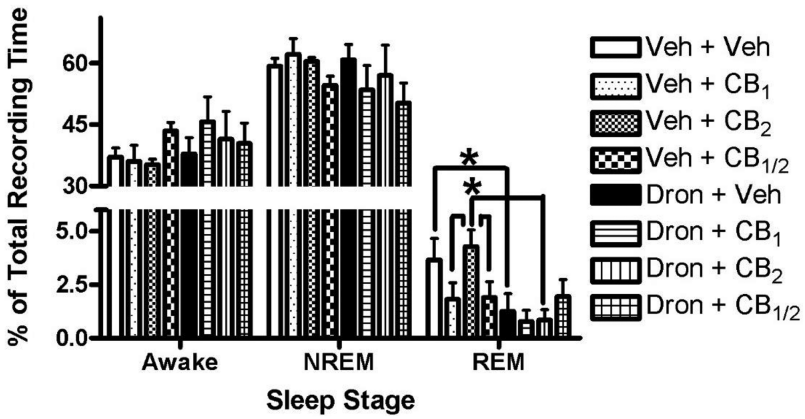


**Figure 2—**Awake time (left), and NREM (center), and REM (right) sleep as a percentage of total recording time quantified from 6-hour recordings of conscious chronically instrumented rat experiments. Vehicle (DMSO in PBS) or dronabinol (10 mg/kg) was injected IP in combination with vehicle (solid bars) or $CB_1$ receptor (AM 251, 5 mg/kg) or $CB_2$ receptor (AM 630, 5 mg/kg) antagonist, or both (shaded bars). Dronabinol and a combination of dronabinol and $CB_2$ antagonist significantly reduced REM sleep. $CB_1$ or combination of $CB_1$/$CB_2$ antagonists also significantly decreased REM sleep compared to $CB_2$ antagonist alone. Data (mean ± SEM) were analyzed using mixed model analysis with repeated/fixed measures (CB agonist and CB antagonist) followed by post hoc multiple comparison tests with Sidak's correction if there were significant main effects or a significant interaction of main effects. *$p < .05$.

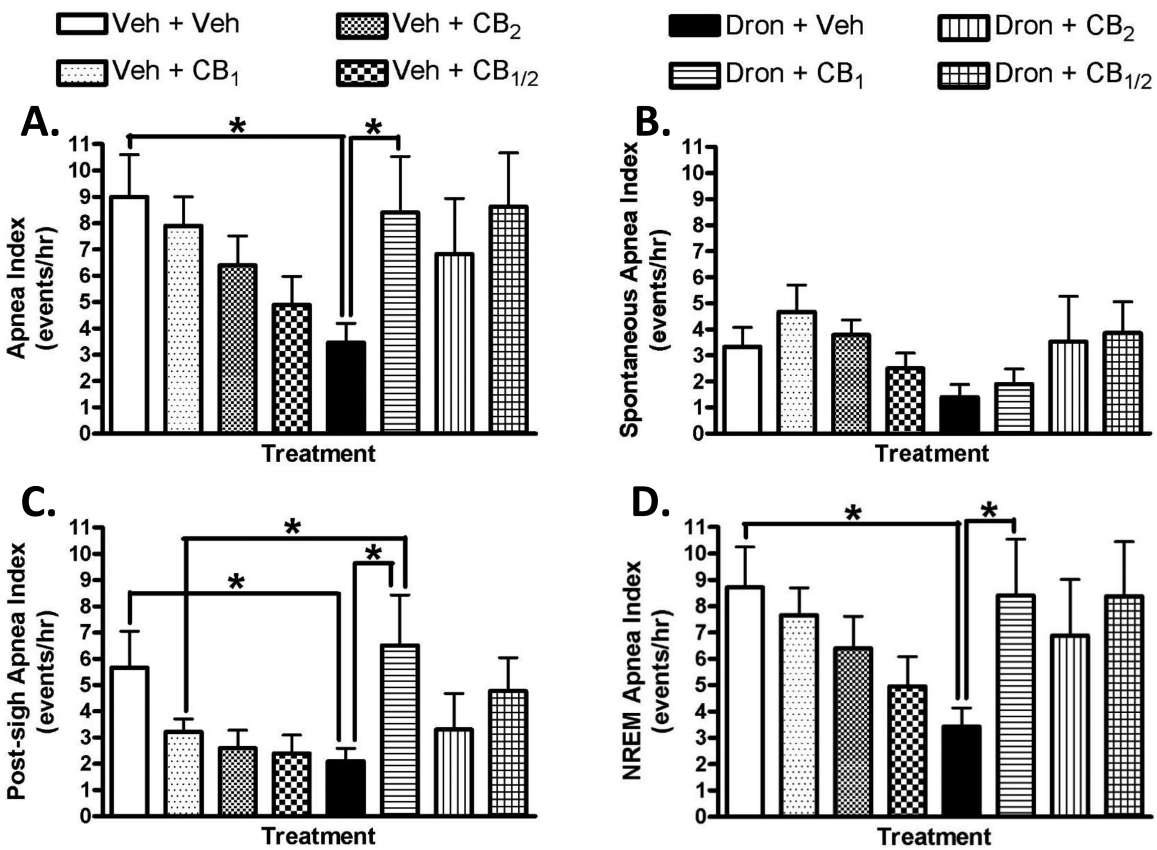


**Figure 3—**Apnea (A), spontaneous apnea (B), post-sigh apnea (C), and NREM apnea (D) indices quantified from 6-hour recordings of conscious chronically instrumented rat experiments. Indices were quantified as events/hour during sleep. Vehicle (DMSO) or dronabinol (10 mg/kg) was injected IP in combination with vehicle (solid bars) or $CB_1$ receptor (AM 251, 5 mg/kg) or $CB_2$ receptor (AM 630, 5 mg/kg) antagonist, or both (shaded bars). Dronabinol significantly decreased the apnea post-sigh, and NREM apnea indices; $CB_1$ antagonism reversed dronabinol's effect. Data (mean ± SEM) were analyzed using mixed model analysis with repeated/fixed measures (CB agonist and CB antagonist) followed by post hoc multiple comparison tests with Sidak's correction if there were significant main effects or a significant interaction of main effects. *$p < .05$.

$N$ = 10) significantly decreased ($p$ < .01) post-sigh apneas compared to vehicle alone (5.66 ± 1.39 events/hour, $N$ = 10). Dronabinol and $CB_1$ antagonist (6.51 ± 1.92 events/hour, $N$ = 8) significantly reversed ($P$ = 0.02) the post-sigh apnea suppression compared to dronabinol alone (2.09 ± 0.50 events/hour, $N$ = 10). Interestingly, $CB_1$ antagonist alone significantly ($p$ = .03) decreased post-sigh apneas (3.21 ± 0.50 events/hour, $N$ = 10) compared to dronabinol and $CB_1$ antagonist (6.51 ± 1.92 events/hour, $N$ = 8), and there was a trend ($p$ < .10) for post-sigh apneas to be suppressed in $CB_2$ antagonist alone treated rats (2.60 ± 0.68 events/hour, $N$ = 10) compared to vehicle controls (5.66 ± 1.39 events/hour, $N$ = 10). Similarly, dronabinol alone (2.11 ± 0.52 events/hour, $N$ = 10) significantly decreased ($p$ < .01) NREM post-sigh apneas compared to vehicle alone (5.73 ± 1.37 events/hour, $N$ = 10). Dronabinol and $CB_1$ antagonist (6.55 ± 1.92 events/hour, $N$ = 8) significantly reversed ($p$ = .03) the NREM post-sigh apnea suppression compared to dronabinol alone (2.11 ± 0.52 events/hour, $N$ = 10). Interestingly again, $CB_1$ antagonist alone significantly ($p$ = .03) decreased NREM post-sigh apnea (3.21 ± 0.50 events/hour, $N$ = 10) compared to dronabinol and $CB_1$ antagonist (6.55 ± 1.92 events/hour, $N$ = 8). To determine if the observed drug-related differences in post-sigh apnea index could be attributable to changes in sigh frequency, we examined the sigh index in each treatment group. There were no significant differences in sigh index between the treatment groups (data not shown).

Apneas during NREM (Figure 3D) sleep followed a similar pattern to that of overall apnea index; there was a significant agonist/antagonist interaction ($F_{3, 48.93}$ = 3.48, $p$ = .02). Post hoc analysis revealed dronabinol alone (3.42 ± 0.72 events/hour, $N$ = 10) significantly decreased apneas ($p$ = .01) compared to vehicle control (8.71 ± 1.54 events/hour, $N$ = 10). Apnea suppression was significantly ($p$ = .03) reversed with dronabinol and $CB_1$ antagonist treatment (8.40 ± 2.14 events/hour, $N$ = 8) and a trend for suppression ($p$ = .09) in dronabinol and $CB_1$/$CB_2$ (8.63 ± 2.04 events/hour, $N$ = 8) compared to dronabinol alone (3.42 ± 0.72 events/hour, $N$ = 10). Post hoc analysis also revealed a trend ($p$ = .07) for $CB_1$/$CB_2$ antagonist alone (4.59 ± 1.09 events/hour, $N$ = 9) to decrease apneas compared to dronabinol and $CB_1$/$CB_2$ antagonist (8.63 ± 2.04 events/hour, $N$ = 8).

## DISCUSSION

The major findings of the present study are: (1) dronabinol decreased REM sleep with no changes in REM bouts or REM bout durations; (2) dronabinol decreased sleep efficiency; (3) dronabinol decreased overall apnea and post-sigh apnea indices; and (4) pretreatment with $CB_1$, but not $CB_2$, receptor antagonist blocked apnea suppression by dronabinol.

These findings were demonstrated using a natural animal model of spontaneous central sleep apnea characterized by us and others.[19] Cessation of breathing during sleep is a result of dynamic interactions between peripheral and central respiratory networks.[21] It is possible that the mechanisms underlying OSA syndrome in humans and sleep-related central apnea in rats may be different. However, both central and obstructive apneas reflect, at least in part, dysregulation of central neural motor output patterning to the respiratory system, including the upper airways.[21] In humans with upper airways predisposed to collapse by anatomical, mechanical, or muscular factors, this dysregulation may be manifested primarily by obstructive apneas.[23] In humans or rats with mechanically stable upper airways, dysregulation of respiratory motor output patterning may be expressed primarily by central apneas or hypopneas.[21] Because their hyoid bone is fixed, rats have mechanically stable upper airways and exhibit central apneas.[12] Viewed in this way, factors that stabilize the pattern of respiratory drive to the pump and upper airway muscles during sleep (eg, reducing high or fluctuating vagal afferent feedback) may have the potential to reduce or eliminate apnea.[7] In fact, overweight/obese individuals without apnea have a moderately compromised upper airway compensated with increased upper airway activation to avoid OSA compared to overweight/obese individuals with OSA.[24] Thus, investigating mechanisms of unstable respiratory patterning in sleeping rats may be expected to yield insights into the pathogenesis of OSA in patients. Empirical support for this perspective derives from the observation that 2 different pharmacological approaches—cannabimimetic and serotonergic—were first demonstrated to reduce central apneas in rats[1,16,25] and subsequently shown to improve OSA syndrome in patients.[14,26]

Dronabinol, a synthetic version of Δ9-tetrahydrocannabinol, is a lipophilic substance that dissolves in sesame oil. To dilute dronabinol to appropriate concentrations for IP injections, DMSO was used[1] to increase absorption across biological membranes and bioavailability of the lipophilic drug.[27–29] DMSO is widely distributed throughout the body, including the brain,[30,31] and is known to affect the blood–brain barrier.[32] DMSO itself has physiological effects, including, for example: decreasing axonal transport in in vitro experiments of the vagus nerve,[33] increasing muscle tone via inhibition of cholinesterase,[34] and modulating morphine-induced nociception.[35] More importantly, DMSO-injected IP modified sleep architecture in rats.[36] To reduce the physiological effects of DMSO, we initially diluted dronabinol in a 25%:75% solution of DMSO:PBS. The only measured effect of dronabinol in this vehicle formulation was reduced REM sleep, with no impact on sleep apneas (data not shown). This was in contrast to previously published experiments from our lab in which 100% DMSO was used to dissolve dronabinol.[1] Due to this disagreement, another set of experiments using dronabinol in 100% DMSO was completed, and not only was there reduced REM sleep (Figure 2) but dronabinol in 100% DMSO also significantly reduced apneas (Figure 3), similar to aforementioned study.[1] There were no differences in apnea frequency between 100% DMSO alone or 25% DMSO:PBS, and apnea frequency for each of these conditions was similar saline-injected rats as previously reported.[1] Thus, DMSO did not artificially increase apneas, and the decrease in apneas observed with dronabinol in 100% DMSO could be attributed to increased bioavailability of dronabinol.

Exogenous nonspecific CBs have been shown to decrease REM sleep in humans[3] and in rats,[1,2] and $CB_1$ receptor signaling has been shown to play a role in REM sleep[2,37,38] and NREM sleep.[38,39] However, other studies failed to demonstrate any effect of altered $CB_1$ signaling on NREM[2] or REM sleep.[39] In this study, dronabinol yielded a decrease in REM sleep. Interestingly, $CB_1$ antagonism without dronabinol also decreased REM sleep,

as previously reported by Goonawardena *et al.*, who hypothesized that $CB_1$ antagonism-induced decreases in REM sleep may be caused by inhibition of $CB_1$-dependent modulation of GABAergic activity in sleep-relevant centers of the brain.[2] As we report here in rats, that same group also observed a lack of reversal of REM sleep suppression in mice treated with a combined treatment $CB_1$ agonist and antagonist, and they hypothesized that the $CB_1$ antagonist is mediating its effect via a CB receptor-independent pathway.[2] Our data show no effect of $CB_1$ or $CB_2$ antagonists on dronabinol-induced decreases in REM sleep. It is known that CBs can allosterically modulate many ionotropic receptors, including serotonergic, glutamatergic, and cholinergic receptors.[40] It is possible that CBs can decrease the activity of cholinergic REM-on neurons causing decreases in REM sleep.[2,41] Further studies will be needed to tease out potential receptor-independent mechanisms of CB modulation of sleep stages.

Dronabinol had a mild impact on sleep efficiency (Figure 1). Though the effects of CBs on sleep efficiency are mixed in human studies,[14,42] we saw a small but significant main effect of CB agonist decreasing sleep efficiency. This effect was not reversed by antagonist treatment and may reflect the fact that dronabinol decreased REM sleep (Figure 2).

Dronabinol had a significant effect on apnea expression (Figure 3). We have previously shown dronabinol's capability in suppressing sleep apneas in rats.[1] Here, we replicate (Figure 3A) and extend this finding, demonstrating that dronabinol's suppression of apneas is driven primarily by $CB_1$ receptor activation. This observation pairs well with the observation that knockout mice lacking the $CB_1$ receptor showed increased apneas.[6] Together, these findings argue that $CB_1$ receptor signaling is important for respiratory stability. $CB_1$ receptors are located in many peripheral and central locations relevant to respiratory pattern generation and motor output integration,[43,44] including the nodose ganglia, the solitary tract,[45] and the hypoglossal motor nucleus,[46] and activation of these receptors can modulate respiratory stability. Though the exact location(s) of $CB_1$ modulation most relevant to apnea suppression cannot be deduced from the present experiments, our previous work implicated modulation of vagal afferents in the genesis/suppression of reflex apneas in anesthetized animals.[4,5] These previous experiments also failed to identify any role for modulation of reflex apneas by global activation of CNS CB receptors[47]; however, we cannot rule out if microinjection into these central local respiratory circuits containing CB receptors, like the solitary tract or hypoglossal nuclei, would have any effect on apnea suppression. Similar to $CB_1$ receptors, $CB_2$ receptors are located centrally in the brainstem,[48] and peripherally on vagal afferents[45] where they modulate reflex apneas.[4] Although we cannot rule out a contribution by $CB_2$ receptors, in the present study, apnea suppression was not driven significantly by $CB_2$ activation.

Why $CB_1$ antagonism reversed dronabinol's suppression of post-sigh apnea, but $CB_1$ antagonism by itself tended to decrease post-sigh apneas needs to be further explored (Figure 3C). Possible contributing factors include at least: differential expression of CB receptors at various sites within the brainstem respiratory circuitry,[45,48,49] the fact that the agents employed (AM251 and AM630) can act as inverse agonists rather than pure antagonists[50] or directly potentiate non-CB receptors (AM251 potentiates $GABA_A$ receptors),[51] and dronabinol's ability to allosterically modulate non-CB receptors.[52] For example, glutamate inhibition has been shown to decrease post-sigh apneas in conscious rats,[53] and CBs can decrease glutamate signaling via allosteric modulation.[52] Recently, it has been shown that the glutamatergic neurons[54,55] of the retrotrapezoid nucleus/parafacial respiratory group play an important role in sigh induction.[56] Moreover, apneas following sighs may be caused by reflex inhibition of inspiration via vagal stimulation from stretch receptors.[21,22,57] However, the effects of dronabinol and CB antagonists had no effect on sigh frequency (data not shown). Taken together with the decrease in post-sigh apneas, it appears that dronabinol has "uncoupled" apneas from sighs. Other works have shown neural correlates for sigh-apnea coupling and the role it might play in the development of sleep apnea.[22,58]

In rat, spontaneous apnea frequency is higher in REM sleep, and post-sigh apnea frequency is similar in NREM and REM sleep.[9–13] This difference, once again, has been attributed to differential control within the brainstem of these two types of apneas.[21,22] In our study, dronabinol decreased REM sleep to such an extent that determining REM apnea index was not possible (Figure 2). Thus, only total apneas and NREM apneas were quantified (Figure 3), and followed the pattern in which post-sigh apneas predominated in NREM sleep (data not shown), though spontaneous apneas also occurred during NREM sleep (data not shown). Our data remain equivocal if decreasing REM sleep leads to decreased apneas.

A final consideration of dronabinol's effects on apnea is that dronabinol may consolidate sleep by increasing low-frequency spectral power.[59] Previous work has shown that certain drugs that decrease apneas also consolidate sleep, reflected by increased EEG delta power during NREM sleep.[60] This may contribute to apnea suppression.[61,62] Though we observed no changes in NREM sleep as a percentage of total sleep, and no changes in sleep/REM/NREM bouts or bout durations, increased low-frequency EEG and deeper NREM sleep may be an explanation for decreased apneas.

A shortfall of this study is that it does not define a mechanism of action of CB-induced stability of breathing. Though this study replicates earlier findings of our lab that CBs modulate apneas in rats,[1] and that modulation of apneas is primarily through $CB_1$ receptor signaling, we cannot completely rule out the participation of $CB_2$ receptor signaling or allosteric modulation of non-CB receptors. More importantly, we cannot isolate if the apnea modulation is occurring peripherally, centrally, or a combination of the two. The use of CB agonists and antagonists that do not cross the blood–brain barrier, the use of intracerebroventricular or brainstem microinjections, and the use of CB agonists specific for $CB_1$ or $CB_2$ receptors may elucidate the mechanisms of CB-induced modulation of apneas. Also, only a single (10 mg/kg) dose of dronabinol was employed, based on our previous findings.[1] This dose, however, was clinically relevant, as a 10 mg/kg intraperitoneal dose leads to a peak plasma concentration in rats[63] that is similar to the peak concentration yielded by a 10 mg total oral dose in human.[64] Lastly, systemic administration of exogenous CBs in rats changes brain wave activity,[59] decreases locomotor activity,[65] and lowers body

temperature, which was blocked by $CB_1$ antagonism.[66] These co-variates may affect sleep patterns and/or propensity for apneas.[67,68] Further research needs to be completed to understand CB administration and these co-variates. Future studies will focus on knocking down CB receptors in rats and studying the effects of dronabinol in these rats.

In conclusion, we show that dronabinol, a synthetic nonspecific CB receptor agonist, decreases REM sleep in a manner that is CB receptor-independent. The present findings also support the conclusion that dronabinol's effects on apnea are mediated at least in part via a $CB_1$ receptor-mediated effect, but the exact mechanism(s) need further clarification. Dronabinol already has been shown to decrease apnea–hypopnea index in humans,[14] and has the potential to become a pharmacotherapy for OSA, providing additional motivation for future studies to clarify the exact mechanisms. More importantly, CB agonists that specifically target $CB_1$ receptor activation may be a novel pharmacotherapy for OSA.

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## DISCLOSURE STATEMENT

Michael W. Calik, PhD, has no conflicts of interest to disclose. David W. Carley, PhD, has conflicts of interest: inventor on intellectual property licensed by the University of Illinois at Chicago to RespireRx (formerly Cortex Pharmaceuticals); stock/stockholder of RespireRx.