

Research report

Monoaminergic and cholinergic modulation of REM-sleep timing in rats

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Abstract

The effects on sleep structure of systemic administration of benchmark cholinergic, serotonergic, and noradrenergic antagonists (QNB, ritanserin, metergoline, and prazosin) were characterized in rats using a new technique for identifying transitions (NRTs) from non-REM (NREM) sleep to REM sleep. In agreement with previous studies, all agents tested reduced REM-sleep expression (by 36–86%). In addition, the serotonergic and noradrenergic antagonists reduced NRT frequency (by 58–81%). The cholinergic antagonist QNB had no effect on NRT frequency. These findings suggest that blockade of serotonergic or noradrenergic receptors increases the interval between REM-sleep episodes, perhaps reducing the rate of accumulation of REM-sleep propensity. Blockade of cholinergic receptors, by contrast, decreases REM-sleep-expression by interfering with REM-sleep maintenance, not by modulating REM-sleep timing. These conclusions are contrary to the predictions of a number of published models of REM-sleep timing.

Keywords: Sleep cycle; State transition; Fourier analysis; Acetylcholine-pharmacology; Serotonin-pharmacology; Norepinephrine-pharmacology

1. Introduction

Rapid-eye-movement (REM)-sleep is promoted by increases in cholinergic activity and/or decreases in noradrenergic/serotonergic activity in the central nervous system [8,9,20,26,27]. Furthermore, cholinergic neurons in the brainstem appear to be maximally active during REM sleep [11,29], while noradrenergic and serotonergic neurons are least active in REM sleep [2,21,22,30]. These findings have encouraged a number of hypotheses according to which acetylcholine, serotonin, and/or norepinephrine control REM-sleep timing (reviewed by [16]).

The above findings do not, however, necessarily entail involvement of acetylcholine, norepinephrine, and serotonin in control of REM-sleep timing. Increases in REM-sleep duration can result from either changes in REM-sleep timing or changes in REM-sleep maintenance. For example, REM-sleep duration is highly sensitive to environmental disturbances. Changes in temperature [15], exposure to an unfamiliar environment [1], and a range of pharmacological treatments [31] reduce REM-sleep duration, pre-

sumably by interfering with REM-sleep maintenance. Treatments that increase REM-sleep duration could act by suppressing the REM-sleep interrupting effects of environmental disturbances.

We have developed a technique for analyzing sleep structure in rats by identifying transitions from non-REM (NREM) sleep to REM sleep (NRTs) based on the EEG phenomena of the pre-REM-sleep stage of NREM sleep [7]. This technique distinguishes between effects on REM-sleep timing and REM-sleep maintenance better than analyses of sleep structure that are based solely on the occurrence of consolidated REM-sleep episodes (discussed in [7]). Changes in REM-sleep timing (speeding or slowing of the NREM-REM cycle) are quantified by changes in the frequency of transitions to REM sleep (per hour of NREM sleep). Effects on REM-sleep maintenance are recognized by changes in the proportion of sustained vs. brief REM-sleep episodes (for details, see [7]).

We here report the effects on sleep structure of systemic administration in rats of the non-specific muscarinic cholinergic antagonist quinuclidinyl benzilate (QNB), the non-specific serotonergic antagonist metergoline, the 5HT₂/5HT_{1C}-selective serotonergic antagonist ritanserin, and the alpha-1 noradrenergic antagonist prazosin. The various hypotheses reviewed in [16] postulate that REM-

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sleep onset is promoted (i.e., the NREM-REM cycle is accelerated) by increased cholinergic activity and/or decreased serotonergic/noradrenergic activity. They would therefore predict that the cholinergic antagonist QNB should decrease the frequency of REM-sleep transitions (retarding REM-sleep timing) and that the serotonergic and noradrenergic antagonists should increase the frequency of REM-sleep transitions (accelerating REM-sleep timing).

2. Materials and methods

Adult male Wistar rats, weighing 250–310 g at time of surgery were instrumented with epidural EEG and nuchal EMG electrodes under anesthesia (ketamine 80 mg/kg, xylazine 8 mg/kg, and acepromazine maleate 1.6 mg/kg administered intraperitoneally with intramuscular supplements as necessary). Details of EEG implantation procedures are described elsewhere [7]. Animals were given an intramuscular 0.25 ml gentamicin antibiotic injection after surgery and monitored until recovery from anesthesia.

Animals were allowed at least 7 days recovery from surgery and were then acclimated to the recording environment for at least 72 h. Animals were housed individually in transparent Plexiglas cages within electrically grounded Faraday cages. One EEG electrode was used to ground the animal. This set-up allowed EEG recording with negligible movement artifact. The recording room was sound-attenuated, dimly lit (lights on 12 h per day, between 08.00 and 20.00), and maintained at 22–25° C. Animals were given food and water *ad lib*.

Animals were cabled via a commutator to a Grass polygraph. Frontal-parietal EEG from one hemisphere was filtered at 0.3 Hz and 35 Hz, digitized at 100 Hz and stored in 10-s epochs on a PC. EEG was Fourier analyzed in 10-s epochs and spectral power was averaged in three frequency bands: delta (0.5–4 Hz), theta (6–9 Hz) and sigma (10–14 Hz). EMG was full-wave rectified, integrated with a 0.5 second time-constant, and stored as one value (0–100) per epoch. Data were collected 24 h per day

except for one hour every 4–8 days for backing up data from the computer's hard disk. Sleep/wake states and transitions from NREM sleep to REM sleep (NRTs) were scored algorithmically according to the above electrographic indices as described previously [7]. Animal-specific thresholds for scoring sleep/wake states and NRTs were determined based on examination of baseline data. In all cases, post-treatment EEG data were examined to ensure that drug effects on EEG indices did not affect state and NRT scoring, thereby producing artifactual changes in sleep structure.

±-QNB (RBI), ritanserin (RBI), metergoline (RBI), and prazosin (SIGMA) were administered i.p. in a vehicle of 0.1 ml DMSO. The above volume of DMSO was determined by control trials to have no effect on sleep parameters (see Table 1). All treatments were administered at the beginning of the rest period (± 30 min). When successive treatments were administered to a given animal, treatments were spaced at least 96 h apart and treatment order was randomized.

All statistical tests were calculated using SAS, v. 6.04. Two-way ANOVAs (treatment \times time-course) were calculated with repeated measures for the time-course variable. All post-hoc tests were Fisher's LSD tests, using significance thresholds of $P < 0.05$. Statistical calculations in Table 1 are two-tailed Student's *t*-tests (treatment vs. vehicle).

3. Results

Sleep structure following vehicle treatment was comparable to normal sleep during baseline recordings (see Table 1). Total sleep time, REM sleep time, and NRT frequency were not significantly different from values in baseline recordings. All drug treatments reduced REM-sleep expression by 36–86% versus vehicle control treatment (see Table 1). Noradrenergic and serotonergic antagonists also reduced NRT frequency (by 58–81%) but the muscarinic cholinergic antagonist QNB had no effect on NRT fre-

Table 1
Sleep state percentages and NRT frequency following administration of cholinergic and monoaminergic antagonists

Treatment	NRT frequency	REM sleep	REM episode duration	Total Sleep Time
Baseline (8)	10.9 (0.7)	12.5 (0.7)	0.9 (0.1)	76.0 (1.3)
Vehicle (6)	11.8 (0.9)	15.4 (2.2)	1.0 (0.2)	79.1 (4.5)
Ritanserin 2.5 mg/kg (5)	3.5 (0.6) ***	6.7 (1.9) *	1.2 (0.4)	78.1 (4.8)
Ritanserin 0.5 mg/kg (4)	4.0 (1.1) ***	9.9 (1.8)	1.6 (0.2)	83.1 (2.8)
Metergoline 1.0 mg/kg (4)	2.2 (0.0) ***	4.5 (1.3) **	1.1 (0.5)	81.2 (3.6)
Prazosin 1.0 mg/kg (5)	4.8 (2.8) *	2.2 (1.2) **	0.2 (0.1) *	69.4 (5.1)
QNB 1.0 mg/kg (5)	10.6 (1.6)	4.3 (1.9) **	0.3 (0.1) *	56.2 (17.2)

All values are expressed as mean (S.E.M.) of the first 4 h following treatment. The number of trials per treatment is given in parentheses after treatment description. NRT frequency is number of NRTs per hour of NREM sleep. NRT means are weighted according to duration of NREM sleep in each trial. REM sleep is calculated as percentage of total sleep time (TST). REM-sleep episode duration is expressed in minutes. TST is calculated as percentage of recording time. Statistics for all treatments calculated vs. vehicle, using two-tailed *t*-test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

quency (see Table 1). QNB and prazosin decreased mean duration of individual REM-sleep episodes (by 69% and 80%, respectively). Total sleep time was not significantly affected by any of the drug treatments.

The effects of ritanserin and QNB were selected for further analysis, to contrast the effects of monoaminergic vs. cholinergic blockade. The time-course of changes in REM-sleep expression following 2.5 mg/kg ritanserin and 1.0 mg/kg QNB are shown in Fig. 1. Both agents significantly reduced REM-sleep time during the period 1-3 h following treatment. Two-way ANOVAs indicated a significant (treatment \times time) interaction for ritanserin ($P < 0.01$) and a significant main effect of treatment for QNB ($P < 0.05$). Because there was no (treatment \times time) interaction effect for QNB, post-hoc tests were not performed.

Ritanserin also reduced NRT frequency for eight h following treatment, whereas QNB had no effect on NRT

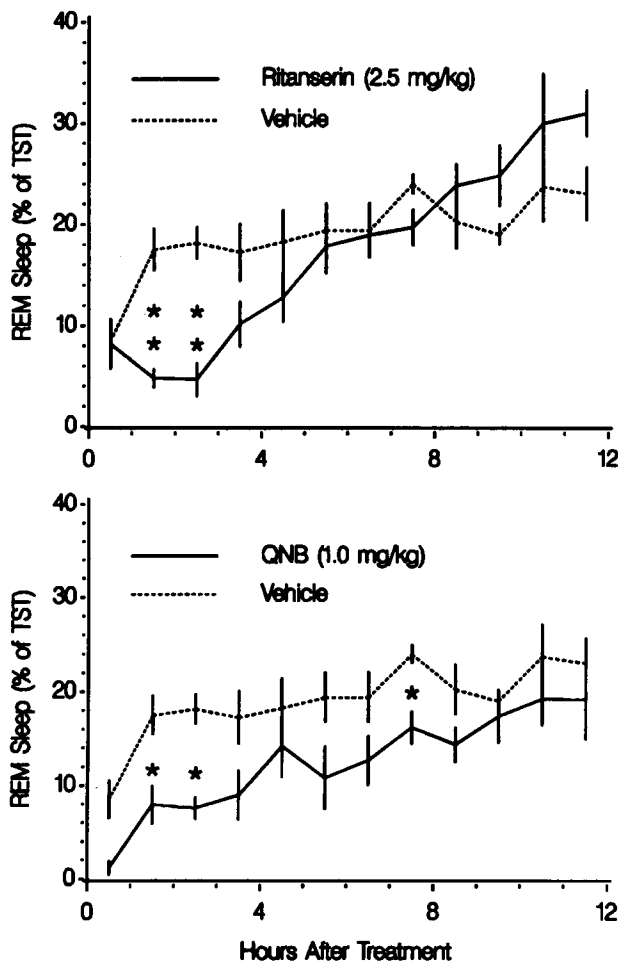


Fig. 1. Time course of changes in REM-sleep expression produced by systemic administration of ritanserin and QNB. REM-sleep expression is plotted as percentage of total sleep time for the first 12 h following administration of 2.5 mg/kg ritanserin (upper panel) and 1.0 mg/kg QNB (lower panel). All values are plotted as mean \pm S.E.M. Because there was no (treatment \times time) interaction effect for QNB (see Results), post-hoc tests were not performed for that treatment. ** $P < 0.01$.

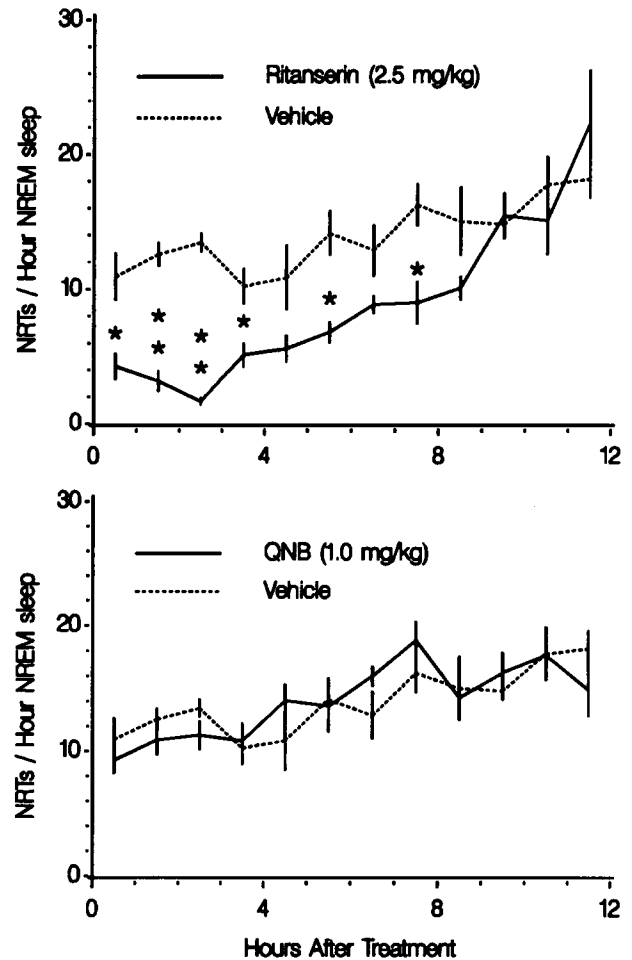


Fig. 2. Time course of changes in NRT frequency produced by systemic administration of ritanserin and QNB. NRT frequency is plotted as the number of NRTs per hour of NREM sleep for the first 12 h following administration of 2.5 mg/kg ritanserin (upper panel) and 1.0 mg/kg QNB (lower panel). All values are plotted as mean \pm S.E.M. Hourly means are weighted by duration of NREM sleep in that hour for each trial. * $P < 0.05$, ** $P < 0.01$.

frequency (see Fig. 2). Two-way ANOVAs indicated a significant main effect of treatment ($P < 0.02$) and a significant (treatment \times time) interaction effect ($P < 0.05$) for ritanserin, and no significant effects for QNB.

The effects of ritanserin and QNB on mean duration of individual REM-sleep episodes is shown in Fig. 3. Ritanserin has no effect on episode duration in the first 4 h and significantly increases REM-sleep episode duration 4-6 h after treatment. QNB decreases REM-sleep episode duration for 8 h following treatment. Two-way ANOVAs indicated a significant main effect of treatment for QNB ($P < 0.05$) and a significant (treatment \times time) interaction effect for ritanserin ($P < 0.02$). Because there was no (treatment \times time) interaction effect for QNB, post-hoc tests were not performed.

An example of sleep structure during following 0.5

mg/kg ritanserin (compared to a time-matched baseline recording from the same animal) is shown in Fig. 4. NRTs are associated with sharp decreases in EEG delta power together with increased EEG theta and sigma activity. In normal sleep, NRTs occur at 4-8-min intervals throughout NREM sleep (see Fig. 4A). Following ritanserin administration, NRT frequency was markedly reduced, as evidenced by long intervals of high EEG delta power in NREM sleep (see Fig. 4B). When REM-sleep episodes occurred, however, the transition to REM-sleep was associated with the same EEG phenomena as in vehicle recordings, indicating that ritanserin did not interfere with expression of the electrographic markers of NRTs. The long periods of sustained, high delta-power NREM sleep (up to 50 min) that occurred following ritanserin treatment, are unparalleled in the rat. In normal rat sleep, the occurrence

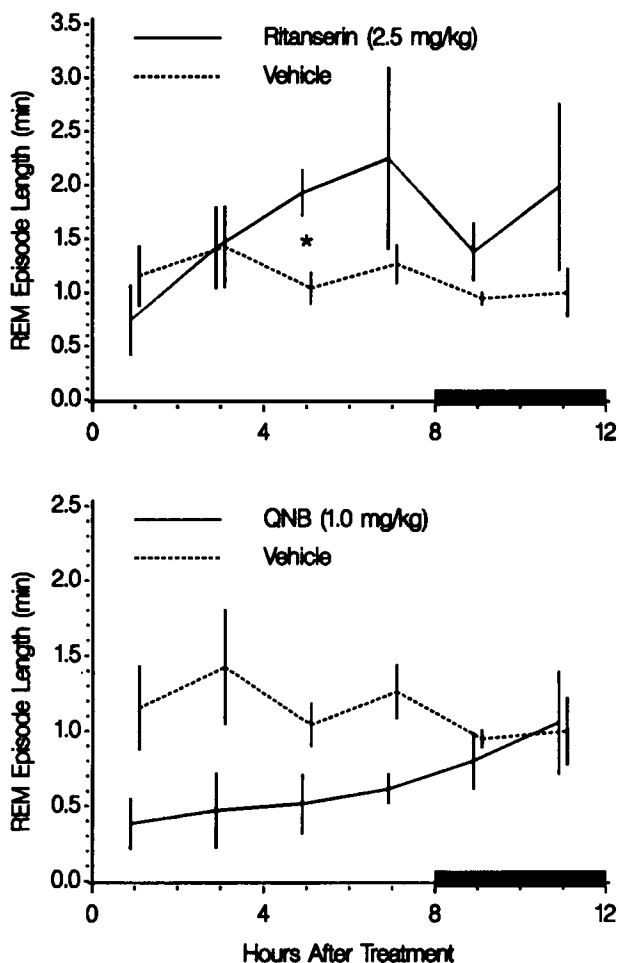


Fig. 3. Time course of changes in mean duration of individual REM-sleep episodes produced by systemic administration of ritanserin and QNB. Mean duration of individual REM-sleep episodes is plotted in minutes, following administration of 2.5 mg/kg ritanserin (upper panel) and 1.0 mg/kg QNB (lower panel). All values are plotted as mean \pm S.E.M. Because there was no (treatment \times time) interaction effect for QNB (see Results), post-hoc tests were not performed for that treatment. * $P < 0.05$.

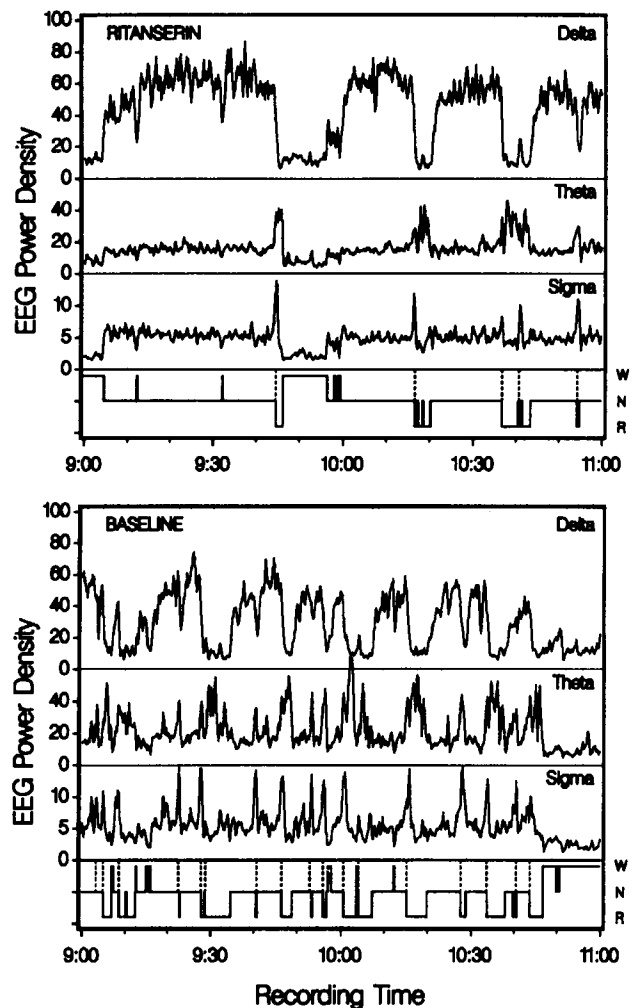


Fig. 4. Examples of EEG power in delta, theta, and sigma frequency bands during 2 h of baseline recording and after systemic administration of ritanserin. Power in each frequency band is plotted in separate windows within each panel. Power values are μV^2 , smoothed using 30-s running averages to facilitate display. The bottom window within each panel shows a sleep/wake state histogram (labeled at right). NRTs are indicated by vertical dashed lines. Both examples are recordings of the same animal. The recording shown in Fig. 3B follows systemic administration of 0.5 mg/kg ritanserin at 8:00.

of more than 10-15 min of NREM sleep between NRTs is unusual [6].

4. Discussion

In agreement with previous studies, we have found that selective blockade of either cholinergic, serotonergic, or noradrenergic receptors inhibits REM-sleep expression (pre-1990 studies reviewed by [31]; more recent studies include [14,17,18,23,25,28,32]). In addition, blockade of serotonergic or noradrenergic receptors reduced the frequency of transitions to REM sleep (NRTs), producing a 'slowing' of the NREM-REM cycle. Blockade of muscarinic cholinergic receptors by QNB did not affect NRT

frequency but decreased the mean duration of individual REM-sleep episodes, suggesting that inhibition of cholinergic receptors reduces REM sleep expression by interfering with REM-sleep maintenance rather than by modifying REM-sleep timing.

These findings are inconsistent with the various cholinergic/monoaminergic models of REM-sleep timing reviewed in [16]. According to those hypotheses, the timing of REM-sleep onset should be advanced by increases in cholinergic and/or decreases in monoaminergic activity. We have found no relationship between decreased cholinergic activity and REM-sleep timing, and decreased serotonergic or noradrenergic activity actually retards the timing of REM-sleep onset.

In retrospect, the majority of previously reported effects of changes in cholinergic activity on REM-sleep expression are attributable entirely to cholinergic promotion of REM-sleep maintenance and do not require any cholinergic control of REM-sleep timing. Cholinergic mechanisms are known to contribute to REM-sleep component phenomena (e.g. active inhibition of motor output and sensory input) that contribute to REM-sleep maintenance (reviewed by [24]). The most potent relationship between cholinergic activity and REM-sleep expression concerns local administration of cholinergic agonists in anatomically specific sites in the brainstem (see [4] for references). These treatments presumably promote REM sleep by direct activation of REM-sleep component phenomena and need not entail any modification of REM-sleep homeostasis. The anatomy and physiology of monoaminergic and cholinergic neuronal systems suggest that release of these neurotransmitters is increased and or decreased in a diffuse manner throughout large regions of the nervous system [3,12,19]. Therefore, systemic administration of monoaminergic and cholinergic agents probably more closely parallels endogenous alterations in release than local administration does.

The findings that have most directly argued for a cholinergic role in REM-sleep timing are that intravenous infusion of the cholinergic agonist arecholine or the acetylcholinesterase inhibitor physostigmine after sleep onset reduce REM-sleep latency in humans, and infusion of scopolamine increases REM-sleep latency [13,26,27]. However, these experiments employed the common criterion in human sleep studies that a REM-sleep episode is not recognized as such unless it is at least 5 min in duration. When this criterion is used, brief REM-sleep episodes are necessarily excluded from the analysis [7]. Skipped and brief first REM-sleep episodes are frequently seen in human recordings [10]. When special criteria are not employed to identify skipped or brief first REM-sleep episodes, REM-sleep latency is artifactually increased. In light of the present findings, we hypothesize that arecholine and physostigmine improve REM-sleep maintenance in the above-described studies, resulting in fewer first REM-sleep episodes that do not meet the 5-min

criterion. It is important to note, however, that the present study utilizes only a cholinergic antagonist and does not categorically exclude a true REM-sleep inducing effect of cholinergic agonists.

The dramatic reductions in NRT frequency produced by serotonergic and noradrenergic antagonists are previously unreported and have important implications for our understanding of REM-sleep timing. We have hypothesized that REM-sleep timing is controlled by accumulation of REM-sleep propensity in NREM sleep [5]. The apparent slowing of the NREM-REM cycle produced by these treatments, together with a reduction in REM-sleep expression, suggest that inhibition of serotonergic and/or noradrenergic receptors reduces the rate of accumulation of REM-sleep propensity, thereby reducing the accumulated need for REM sleep.

Both noradrenergic and serotonergic antagonists reduce NRT frequency. However, the effects of prazosin, the alpha-1 noradrenergic antagonist used in this study are somewhat ambiguous. Unlike ritanserin and metergoline, prazosin appears to interfere somewhat with expression of the electrographic phenomena by which we score NRTs. Moreover, prazosin treatment, unlike ritanserin (see Fig. 3), does not produce long periods of uninterrupted, high delta-power NREM sleep. The actual frequency of transitions to REM-sleep following prazosin treatment may therefore be higher than the value we have reported.

The fact that NRT frequency is reduced by the selective 5HT-2/5HT-1C antagonist ritanserin suggests that serotonergic modulation of REM-sleep timing is 5HT-2/5HT-1C mediated. This is especially interesting in light of the fact that 5HT-2 and 5HT-1C receptors in adult rats are localized in the forebrain but not in the brainstem, where REM-sleep timing has been generally thought to be controlled.

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