

EFFECT OF AGE IN SEXUAL BEHAVIOR OF SLEEP DEPRIVED RATS AFTER COCAINE ADMINISTRATION

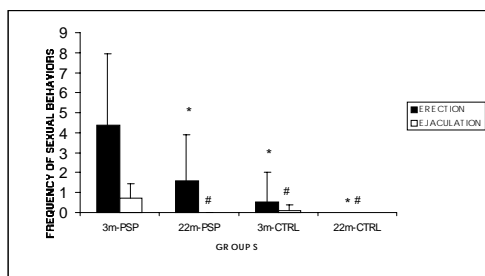
Monica Levy Andersen; Magda Bignotto, Ricardo Borges Machado; Sergio Tufik
Department of Psychobiology – Universidade Federal de São Paulo – UNIFESP, São Paulo, SP, BRAZIL

Introduction: It has been suggested that paradoxical sleep deprivation (PSD) could sensitize dopaminergic systems, probably by inducing supersensitivity of the post synaptic receptor (TUFIK et al., 1978). The mesolimbic dopamine (DA) system, composed of DAergic neurons in the ventral tegmental area and their projections to the nucleus accumbens and other forebrain structures, has been implicated in the reinforcing and locomotor-activating properties of cocaine (HOGER et al., 1999). In 2000, ANDERSEN et al., showed that acute cocaine administration elicited sexual behavior in young PSD rats. It is known that there is a decline in sexual arousal and copulatory activity in male rats with advancing age, as well as significant changes in various sleep parameters. Thus, the objective of the present study was to examine the behaviors of penile erection and ejaculation in young and old-age rats submitted to PSD after cocaine administration.

Methods: Young (3 months) and middle-age (22 months) male rats were PSD for 96 hours by the modified multiple platform methods or stayed in their cages (control group-CTRL). At the end of this period, the animals received an i.p. injection of cocaine (15 mg/kg) and the erections and ejaculations were assessed for 60 minutes.

Results: The cocaine-PSD study revealed a significant difference between the 3months-PSD and the three other groups with regard to sexual behavior. The young PSD rats had the greatest number of erection and ejaculation. Among the old-age rats, the PSD group only had erection and sexual behavior was absent in the 22 months control group.

Table 1:



* # - different from 3m-PSD animals; p<0,0005 by ANOVA test

Conclusions: Sexual function commonly decreases with age, but the interaction of sleep deprivation and the action of cocaine in the brain by enhanced DA transmission can facilitate and increase the sexual behavior in old-age rats.

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HIGH-AFFINITY [³H]-OUABAIN BINDING TO THE Na⁺/K⁺-ATPase FROM DISCRETE BRAIN REGIONS OF REM SLEEP-DEPRIVED RATS

Marco A. C. Bedito, Umberto Andrade and José Gilberto B. Carvalho
*Departamento de Psicobiologia, Universidade Federal de São Paulo,
 São Paulo, Brasil*

REM sleep deprivation increases brain excitability (Physiol. Behav. 7: 103-106, 1971). Na⁺/K⁺-ATPase has a critical role in restoring transmembrane gradients of Na⁺ and K⁺ after neuronal excitation (Amer. J. Physiol. 279: C541-C566, 2000). Ouabain, a Na⁺/K⁺-ATPase inhibitor, binds to the phosphorylated catalytic subunit of Na⁺/K⁺-ATPase with a 1:1 stoichiometry, providing and estimation of the number of Na⁺/K⁺-ATPase molecules. Three Na⁺/K⁺-ATPase isoforms are expressed throughout the brain regions in a great complexity. These enzyme isoforms differ in their affinity to ouabain, a Na⁺/K⁺-ATPase inhibitor. The aim of this work was to determine the high-affinity binding of [³H]-ouabain to Na⁺/K⁺-ATPase from discrete brain regions of rats deprived of REM sleep (REMSD). Adult, male, Wistar rats were deprived of REM sleep for 96h by the flower-pot technique. A home-cage and a large platform group (stress control group) were also used. [³H]-Ouabain binding assays were carried out in a crude membrane preparation (30,000 g pellet) from striatum (40 nM), hippocampus (68 nM) and brainstem (60 nM). One-way ANOVA detected a statistically significant difference in [³H]-ouabain binding (pmol/mg protein, mean ± SD) in all brain regions assayed (striatum: F_{2,15}=5.7, p=0.01; hippocampus: F_{2,15}=6.3, p=0.005; brainstem: F_{2,13}=15.3, p=0.0004). Post-hoc statistical analyses showed that both REMSD and stress control group differed significantly from home-cage group (*p<0.01, two-tailed).

	Striatum	Hippocampus	Brainstem
Home-cage	2.7±0.9 (5)	6.8±1.3 (6)	15.3±0.4 (4)
Stress control	4.7± 0.9* (6)	9.8±2.0*(6)	19.3±1.8* (5)
REMSD	4.5±1.3* (7)	9.2±1.1* (6)	20.7±1.7*(7)

(N) number of rats

The results suggest an increase in Na⁺/K⁺-ATPase molecules in the brain areas assayed. The increase may be due to stress or may indicate that stress control group was also deprived of REM sleep.

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EFFECT OF MIRTAZAPINA IN DEPRESSIVE PATIENTS WITH INSOMNIA

Francesca Canellas and José Maria de Pedro

Psychiatry Department, Hospital Son Dureta, Palma de Mallorca, Spain

Introduction: Depressive disorders are severe and prevalent illnesses, which can be considered an important public health problem. Sleep disorders are integral component of depressive disorders and approximately 95% of depressive patients suffer insomnia. Depressive patients with insomnia have a diminution of cognitive and motor performance with higher impairment in life quality than the rest of depressive patients.

Objective: Evaluate the efficacy and tolerance of Mirtazapine in the treatment of depressive disorders associated with insomnia in Primary Health care setting.

Study Desing: This was a 6-week observational, prospective, non controlled, national, multicentric study. Three visits (basal, day 15 and day 45) were performed for each patient. Montgomery-Asberg (MADRS) scale for depression, Spiegel Sleep Questionnaire and an Adverse Events Questionnaire were performed in each visit. All patients with at least a follow up visit were used for efficacy analysis and all patients who took at least one dose of medication for tolerability analysis. All patients received a single nightly dose of 15 mg of Mirtazapine the first four days and 30 mg since the 5th. Practitioners were allowed to adjust the dose in each control visit whiting a range of 15-45 mg/day.

Results: A total of 538 General Practitioners have studied 2064 ambulatory patients with depression and insomnia (DSM IV criteria) that could be included in the study.

In this preliminary report we present the data of the first 131 patients having finished the protocol, 33 men (25,19%) and 98 women (74,81%).

MADRS Scale: Total punctuation of scale was 30,55 (+/-7,76) in the basal visit, 20,61 (+/- 8,94) at the 15 days visit and 9,06 (+/- 7,70) at the final one. Decrease in punctuation was significant ($p < 0,0001$) since the first control.

Spiegel: Patients also improved the sleep quality and duration, being significant the results since the first control. No patient was withdrawn from the study due to lack of efficacy. Nine patients (6,82%) showed adverse reactions being the most usual one the diurnal somnolence (3 subjects, one of them had to be withdrawn). The remaining complaints were: bradypsychia (1), suicide attempt (1), constipation (1), hypotension (1), rash (1) and finally a patient had a non-specified crisis.

Conclusion: Mirtazapine has been shown to be an efficient and well tolerated treatment for depressive patients with insomnia.

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DISTINCTIVE EFFECTS OF MODAFINIL AND d-AMPHETAMINE ON THE HUMAN EEG POWER SPECTRUM DURING 60 UNINTERRUPTED WAKING HOURS

Florian Chapotot¹, Ross Pigeau² and Alain Buguet¹

¹*Unité de Physiologie de la Vigilance, Centre de Recherches du Service de Santé des Armées, France.*

²*Human Factors of Command Systems Section, Defence and Civil Institute of Environmental Medicine, Canada*

Modafinil, a new wake-promoting agent, reduces the need for long recovery sleep [1] and decreases the rebound in EEG slow wave activity following sleep deprivation [2]. These diachronic effects suggest an action of modafinil on a homeostatic sleep-wake regulatory process. The aim of this study was to determine whether modafinil, in comparison to d-amphetamine and placebo, would affect the homeostatic process that also influences EEG activity during waking [3].

Thirty-three healthy subjects were investigated during 60 hours of prolonged wakefulness in a double-blind placebo-controlled mixed-design study. A 4-min eye-closed maintenance-of-wakefulness test was administered hourly allowing the measurement of sleep latency and waking EEG activity. The effects of 300-mg of modafinil and 20-mg of d-amphetamine administered at three different times were evaluated.

Both stimulants increased sleep latency during 10-12 hours following ingestion, independently of the time of administration. At the level of the waking EEG spectrum, d-amphetamine strongly attenuated the natural circadian rhythms and suppressed the sleep deprivation-related increases in low frequency activity (0.5-7 Hz). In contrast, the evening administration of modafinil had little effect on the EEG circadian rhythms and allowed for the maintenance of slow alpha (8.5-11.5 Hz) frequency activity, which exhibited a homeostatic decrease proportional to time awake under placebo.

These findings demonstrate that the wake-promoting action of modafinil and d-amphetamine involves separate neurophysiological regulatory systems: d-amphetamine may inhibit the expression of sleep processes, probably through a direct cortical activation masking circadian rhythms in EEG bands; modafinil, through a synchronic effect, may disrupt the homeostatic process controlling the expression of waking EEG activity.

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SCOPOLAMINE DECREASES RHO BAND (20-30 Hz) EEG POWER DURING REM SLEEP: IMPLICATIONS FOR MEMORY PROCESSES

Oscar Díaz-Ruiz¹, Isabelle Beaulieu¹ and Roger Godbout^{1,2}

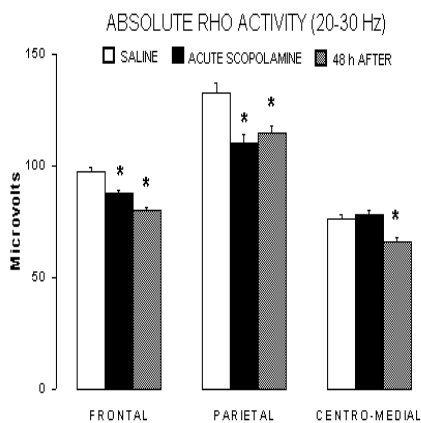
¹Centre de Recherche, Hôpital Sacré-Coeur, ²Département de Psychiatrie, Université de Montréal, Québec, Canada

Administration of the muscarinic receptor antagonist scopolamine acutely decreases REM sleep and impairs memory.¹ Forty-eight hours after treatment, however, no effects on REM sleep are found² while memory impairments are present.³ We used qEEG analysis of REM sleep to evaluate further this apparent dissociation. Special attention was paid to Rho EEG activity, a cortical EEG frequency selectively associated with REM sleep in the rat.⁴

Methods. Long-Evans rats aged 3-4 months were implanted for sleep recordings and EEG electrodes were placed over frontal (A= Bregma+3.5 mm; lat. +1.0mm), parietal (A= Bregma-2.0 mm; lat. + 3.5mm), and centro-medial (midway between Bregma and Lambda; lat. +1.0) cortices, referred to cerebellum. After full recovery, rats received either scopolamine (0.1 mg/kg, s.c.; n=9) or vehicle (0.5 cc NaCl 0.9%, s.c.; n=14) at 8 AM, 2 h after lights on; sleep recordings started immediately, for 4 h. Five of the scopolamine-treated rats were recorded again, 48 h later. Fifteen 4-sec epochs of artifact-free EEG were selected from REM sleep and submitted to Fast Fourier Transform. Absolute power amplitude was calculated and six frequency bands were created: Delta (0.75-3.75 Hz), Theta1 (4-6.75 Hz), Theta2 (7-9.75 Hz), Sigma (10-13.75 Hz), Beta (14-19.75 Hz), Rho (20-30 Hz). Results were compared with t-test for independent samples.

Results. Compared to saline-treated rats, acute scopolamine decreased the percentage of REM sleep (16.3 ± 1.3 vs 7.4 ± 1.1 ; $p < .05$) and increased the percentage of wake after sleep onset (12.7 ± 1.4 vs 55.8 ± 7.0 ; $p < .05$); both measures returned to saline values 48 h later. qEEG analysis showed that acute scopolamine increased all Frontal frequencies except Rho, which was decreased, further decreasing after 48h. Acute scopolamine increased Delta and decreased Rho at the Parietal site while it increased Beta activity and decreased Rho at the Centro-medial site.

Discussion. The reported long-term effects of scopolamine on memory are only paralleled by REM sleep EEG Rho activity. This suggests that common neurophysiological networks may be involved.



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EFFECT OF MODAFINIL (PROVIGIL) ON THE SINGLE-UNIT ACTIVITY OF
SEROTONINERGIC DORSAL RAPHE NEURONS IN BEHAVING CATS

Monica MC Gonzalez, Barry L. Jacobs, Casimiro Fornal
*Laboratory for Neuromodulation & Behavior, VAMC
Philadelphia, Pennsylvania, United States*

Claim of Abstract: Modafinil has non-significant effect on serotonergic neuronal activity.

Abstract: Modafinil {2-[(diphenylmethyl)sulfinyl]acetamide} is a novel wakefulness-promoting agent, used in the treatment of narcolepsy and hypersomnia, which has a pharmacological profile distinct from that of amphetamine and other central nervous system stimulants. Although its mechanism of action is not known, recent evidence suggests that the drug may act, at least in part, by enhancing the release of serotonin in the brain (Ferraro et al., 2000). To further characterize the effects of modafinil on the central serotonergic system, the present study monitored the discharge rate of serotonin-containing neurons in the dorsal raphe nucleus of cats, following systemic administration of this drug. Under pentobarbital anesthesia, adult cats were implanted with insulated nichrome microwires for recording neuronal activity and with electrodes (EEG, EOG, EMG) for monitoring behavioral state, as described previously (Fornal et al., 1999). Administration of modafinil (5 or 10 mg/kg, p.o.) induced a state of continuous wakefulness, lasting 10-18 h, without apparent behavioral excitation or hyperactivity. Cats typically adopted a quiet, recumbent posture with eyes open, during the period of enhanced waking. Serotonergic neuronal activity was not significantly altered from baseline levels at any time after modafinil administration. A slight increase in firing rate (up to 30%) was noted for some cells, however, most cells displayed less than $\pm 10\%$ change in neuronal activity. Overall, these results indicate that the pronounced waking effect observed with modafinil in cats is not accompanied by marked changes in the activity of brain serotonergic neurons.

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SLEEP-WAKEFULNESS EEG ANALYSIS AND CENTRAL NERVOUS SYSTEM
EFFECTS OF TOTAL AQUEOUS EXTRACTS OF *VERVAIN HASTATA*
(VERBENACEAE) IN RATS

Moses A. Akanmu, Kazuki Honda, Shojiro Inoué

*Department of Biocybernetics, Institute of Biomaterials and Bioengineering,
Tokyo Medical and Dental University, Tokyo, Japan*

The sleep-wakefulness EEG analysis and central nervous system effects of the total aqueous extracts (TAE) of aerial portion of vervain hastata (Verbenaceae) was studied in male rats in order to establish the scientific basis in herbal medicines. The plant has been empirically used in the herbal preparations as sedative, relaxant, nerve tonic, analgesic and thymoleptic among other uses, and no data is available. The investigation was conducted using the EEG analysis, barbituric-hypnosis test, acetic acid-induced writhing test and, open-field behavioral study procedures with doses ranging from 125 to 2000 mg/kg per oral (p.o.) or intraperitoneally (i.p.).

In the barbituric-hypnosis test, administration of TAE in three doses of 500, 750, and 1000 mg/kg p.o. prior to an injection of pentobarbital (30 mg/kg i.p.) was carried out and at the same time investigated the involvement of GABA_A-benzodiazepine receptors system by administering a specific antagonist flumazenil (3 mg/kg i.p.) 15 min before the barbiturate-hypnosis test in another group of rats. The positive control group of rats was treated with diazepam (3 mg/kg i.p.). The EEG sleep-wakefulness study was analyzed in individually housed rats, maintained under 12:12 light/dark cycle after the implantation of electrodes in the cortex and neck muscle for EEG/EMG recordings. Continuous 24 h EEG recording was done during baseline day (vehicle only) and experimental periods before and during the administration of TAE. The total aqueous extract was administered via drinking water at concentration of 30 and 50 mg/ml. The anti-nociceptive test was carried out using the acetic acid-induced writhing method.

The results showed that TAE significantly ($p < 0.01$) potentiated the pentobarbital induced-hypnosis by reducing sleep latency and increased sleeping time in a dose-dependent manner. Flumazenil reversed these effects in all animals. Comparison of the EEG data between baseline and extract administration periods showed that TAE augmented total sleep time, time spent in rapid eye movement (REM) sleep and time spent in nonREM sleep at the expense of wakefulness, which were consistent with the barbituric-hypnosis test results. The anti-nociceptive results showed that the extracts possess significant ($p < 0.05$) dose-dependent activity by increasing the pain threshold.

In conclusion, the results clearly show the scientific validity for the use of this plant as a sedative, mild analgesic and possibly as a nerve tonic material.

A DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY OF THE EFFICACY OF TRAZODONE IN THE ALCOHOL POST-WITHDRAWAL SYNDROME: POLYSOMNOGRAPHIC AND CLINICAL EVALUATIONS

Olivier C Le Bon, Guy Hoffmann, James R Murphy, Nicolas Kormoss, Monique Kentos, Philippe Dupont, Karin Lion, Isidore Pelc and Paul Verbanck
Pl. Van Gehuchten 4, Brussels, Belgium

Alcohol detoxification is accompanied by sustained difficulties in sleep initiation and maintenance. These difficulties are thought to be an important cause of relapse to alcohol use. However, the choice of a hypnotic drug is made difficult by cross-tolerance between benzodiazepines and alcohol. We evaluated here the capacity of trazodone, a second generation antidepressant with anxiolytic and sedative properties, to increase the sleep efficiency in alcohol dependent patients after detoxification.

Eighteen patients completed the trazodone (n=8) or the placebo (n=8) treatment arms. Polysomnographies were performed at baseline, after the first drug intake, and after four weeks of treatment. The main outcome variable was sleep efficiency. Secondary outcome measures included changes in other sleep parameters, Hamilton depression rating and clinical global impression scales.

The sleep efficiency was increased in the trazodone group when it was computed after sleep onset, both immediately after drug intake and after four weeks of treatment. No benefit was observed in the placebo group. Sleep improvement under trazodone also included the number of awakenings, intermittent wake sleep time and non-rapid eye movement sleep. Hamilton and clinical global scales were better for the trazodone group. Trazodone is thus an interesting option in the treatment of alcohol post-withdrawal insomnia.

Considering the prevalence of alcohol dependence, the reduced rates of remission and the relapses linked to insomnia, even if a fraction of alcohol-dependent patients with insomnia could be helped, a large number of individuals and their environment could be positively affected

INTRASTRIATAL ADMINISTRATION OF AMPHETAMINE INCREASES THE
C-FOS EXPRESSION IN THE PONTOMESENCEPHALIC TEGMENTUM
NUCLEI AS A POSSIBLE MECHANISM OF INDUCED WAKEFULNESS

Juan Mena and Magda Giordano

Centro de Neurobiología, UNAM, Juriquilla, Queretaro, Mexico

It has been shown that rapid eye movement sleep (REMs) deprivation induces increases in dopamine content in the striatum (Farooqui et al, 1996), and c-Fos expression in pontomesencephalic nuclei (Maloney et al, 1999). Anatomical studies have determined that the GABAergic output neurons of the substantia nigra pars reticulata from the basal ganglia reach the cholinergic neurons of the pedunculopontine tegmental nucleus (PPT) (Grofova and Zhou, 1998). In turn, the PPT projects back to the substantia nigra pars compacta, the subthalamic nucleus and the pallidum (Parent and Hazrati, 1995). It is possible that the basal ganglia nuclei through their interaction with the PPT may participate in the modulation of the sleep-wake cycle. To test the hypothesis that direct dopaminergic stimulation of the striatum resulted in c-Fos expression in the PPT region, unilateral injections of d-amphetamine sulfate (15ug/1ul) were delivered into the left caudate-putamen of male Sprague-Dawley animals under deep anesthesia. In order to allow c-Fos expression, animals were perfused one hour after injection and PPT brain sections were processed by immunohistochemical techniques. The results show that there is an increase in c-Fos expression within the cholinergic population of the PPT after the amphetamine injection. To further support the notion of striatal participation in PPT activation, c-Fos expression was also evaluated after striatal kainic acid lesions; these experiments are in progress.

NEUROTRANSMITTERS AND NEUROMODULATORS INVOLVED IN THE REGULATION OF THE WAKING STATE

Jaime M. Monti and Héctor Jantos

Department of Pharmacology and Therapeutics. Clinics Hospital. Montevideo,
Uruguay

[SYMPOSIUM]

It is generally agreed that normal waking (W) and the functions associated with it depend upon the integrity of the entire brain, but that subcortical structures situated primarily in the brainstem are critical for the EEG manifestations of W. In this respect, neurons within the brainstem reticular formation (oral pontine and midbrain reticular formation) activate the cerebral cortex via the nonspecific thalamocortical projection system, and via the subthalamus, posterior hypothalamus and basal forebrain (nucleus basalis of Meynert, nuclei of the diagonal band, and septum).

Neuroanatomical, neurochemical and neuropharmacological studies, particularly those employing selective norepinephrine (α_1), dopamine (D_1 and D_2), serotonin (5-HT_{1-2,3}) or histamine (H_1) receptor agonists have shown that they tend to promote W and to suppress slow wave sleep (SWS) and REM sleep (REMS). In addition, different glutamate receptors and peptides (substance P, corticotropin-releasing factor, thyrotropin-releasing factor, vasoactive intestinal peptide and neurotensin) have wake promoting effects. On the other hand, acetylcholine (M_1 and M_2 receptors) plays an integral role in EEG arousal and REMS generation. Moreover, experimental evidence tends to indicate that adenosine (A_1 and A_{2A} receptors) and GABA ($GABA_A$ receptor) are involved in the induction of sleep and the decrease of W.

Nitric oxide (NO) is an unconventional neurotransmitter synthesized on demand by the enzyme NO synthase (NOS). NO has been proposed to serve multiple functions in the central nervous system including the modulation of the sleep-wake cycle and circadian rhythms, which may in part be related to its influence on neurotransmitter release. A number of *in vivo* and *in vitro* studies have shown that treatment with NO donors increases the basal release of GABA, adenosine and acetylcholine, and reduces the release of norepinephrine, dopamine, serotonin, and histamine in the mammal brain. A decrease in the availability of NO induces the opposite effects.

The anatomical structures involved in the control of W, NREMS and REMS are reciprocally interconnected, and the alternation of behavioral states could depend upon the predominant release of GABA and adenosine, acetylcholine or catecholamines, serotonin and histamine at critical sites. NO could have a significant role in this respect. Whether a reduction in the availability of NO could explain, at least in part, the increased incidence of W in primary and secondary insomnia is an open question.

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THE EFFECTS OF 8-BROMO-cGMP ON SLEEP IN RATS

Ana C. Ribeiro and Levente Kapás

Department of Biological Sciences, Fordham University, New York, USA

Introduction: Brain levels of cGMP show pronounced circadian variation (1) and cGMP has been shown to play a critical role in circadian entrainment in response to light, glutamate and nitric oxide (NO) in the SCN (2). NO is a neurotransmitter implicated in sleep regulation (3). Since NO is a potent activator of soluble guanylyl cyclase and cGMP mediates many physiological effects of NO, experiments were undertaken to investigate whether cGMP itself modulates sleep.

Methods: Male rats were implanted with EEG and EMG electrode and a cannula targeted at either the lateral cerebral ventricle (icv) or the medial preoptic region (anterior diencephalic microinjection). Sleep was recorded for 23 h starting at dark onset from the rats on a baseline day after a saline injection (5 ml icv and 1 ml diencephalic). On the test day, the rats received 8-Br-cGMP icv [6.4 ng (n = 7), 160 ng (n = 7), 4 mg (n = 11), 100 mg (n = 8), 500 mg (n = 11) or 2.5 mg (n = 5)]; or 400 ng (n = 8) intra-diencephalically. Sleep was recorded for 2-11 days. The 4 mg dose of 8-Br-cGMP icv was also tested at light onset.

Results: Cerebral injection of 8-Br-cGMP suppressed NREMS during the dark phase and transiently increased REMS throughout the recording period. NREMS amounts were decreased by 28.2 ± 8.9 and 48.5 ± 13.5 min in the first and second dark periods, respectively, after the 4 mg dose. There was a strong decreasing trend in EEG power across all frequency ranges and vigilance states. Similar tendencies in sleep amounts and intensity were observed after icv injection of lower and higher doses at dark onset. Light onset injection of 8-Br-cGMP did not elicit any changes in sleep parameters. Diencephalic microinjection of 400 ng 8-Br-cGMP suppressed NREMS by 47.2 ± 18.3 and 52.9 ± 22.7 min on the test day and on the recovery day, respectively. REMS amounts and EEG delta activity during NREMS were slightly increased after diencephalic 8-Br-cGMP.

Conclusion: Cerebral injection of cGMP elicited changes in sleep amounts. The second messenger cGMP may be involved in mediating the effects of other sleep factors, including NO.

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RANDOMIZED, DOUBLE-BLIND CLINICAL TRIAL, CONTROLLED WITH PLACEBO, THE TOXICOLOGY OF CHRONIC MELATONIN TREATMENT

Maria L. Seabra, Magda Bignotto, Luciano R. Pinto Junior and Sergio Tufik
São Paulo, Brazil

Melatonin (N-acetyl-5-metoxytryptamine) is a secretory product of the the pineal gland. The mechanisms by which melatonin exerts its hypnotic effects are a matter of speculation. Some authors believe that this effect results from increased indolamine levels at the beginning of the sleep period, suggesting that endogenous melatonin participates in the regulation of the sleep-wake cycle, leading to a cascade of events that may activate somnogenic structures or that even melatonin metabolites possess a hypnotic effect. However, the existence of a causal relationship between sleep and melatonin is still unclear. Therefore, since melatonin is a natural substance, several studies have proposed its use by humans. Some studies have been carried out to evaluate melatonin's possible toxicity. In the present study, we also examined the possible toxicological aspects of melatonin as well as its effects on sleep in normal volunteers. The safe therapeutic use of melatonin depends on its pharmacological effects and clinical and toxicological results. Therefore, the present study was conducted to evaluated the melatonin effects on biochemical parameters, as well as on sleep-related behavioral aspects, sleep architecture and adverse effects, in healthy volunteers, who ingested 10 mg of melatonin/day for a period of 28 days. Analysis of the PSG showed a statistically significant reduction of stage 1 of sleep in the group melatonin. No other differences between placebo and melatonin groups were obtained. In the present study we did not observe, according to the parameters analyzed, any toxicological effect that might compromise the use of melatonin at a dose of 10 mg for the period of time utilized in this study.

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