Melatonin Increases Following Convulsive Seizures may be Related to its Anticonvulsant Properties at Physiological Concentrations

Author   **A. Molina-Carballo[1], A. Muñoz-Hoyos[1], M. Sánchez-Forte[2], J. Uberos-Fernández[1], F. Moreno-Madrid[2], D. Acuña-Castroviejo[3]**

Affiliation   [1] Departamento de Pediatría, Hospital Universitario San Cecilio, Granada, Spain
[2] Hospital de Baza, Granada, Spain
[3] Instituto de Biotecnología, Universidad de Granada, Granada, Spain

Abstract
▼

Melatonin (*N*-acetyl-5-methoxytryptamine, aMT) is an indoleamine produced by several organs and tissues including the pineal gland. Melatonin (aMT) modulates the activity of the brain, mainly acting on both GABA and glutamate receptors. Previous studies have shown the participation of melatonin in the control of convulsive crises, suggesting that aMT concentration increases during seizures, and that patients with seizures of diverse origins show an alteration of the aMT rhythm. However, what is not known is the duration of the aMT response to seizures, and whether aMT changes during seizures could be a marker of the disease. For this reason, the serum levels of aMT in 54 children with a convulsive crisis, febrile and epileptic, were analyzed during the crisis, as

well as at 1 h and 24 hours after the seizure. The results show that aMT significantly increases during the seizure (Day group, 75.64 ± 45.91 and Night group, 90.69 ± 51.85 pg/mL), with normal values being recovered 1 h later (Day group, 26.33 ± 10.15 and Night group, 27.78 ± 7.82 pg/mL) and maintained for up to 24 hours, when the circadian variation of aMT returns to the normal acrophase. Due to the interindividual variation of aMT levels among healthy people, a single determination of the indoleamine concentration is not a suitable marker of the existence of a convulsive crisis unless the circadian profile of aMT secretion in the patient is known. The results obtained also support the view that the stimulation of aMT production by the convulsive crisis may participate in the response of the organism against the seizures.

Correspondence
Dr. A. Muñoz-Hoyos
Departamento de Pediatría
Facultad de Medicina
Avenida de Madrid 11
18012 Granada
Spain
Tel.: +34/958/023 99 6
Fax: +34/958/240 74 0
amunozh@ugr.es

Introduction
▼

Among the best known effects of melatonin (aMT) is its capacity to modulate the central nervous system [4]. The neuromodulatory activity of aMT arises from its two main actions, that of rhythm synchronizer, involving the regulation of the rhythms of both neurotransmitters secretion and receptors, and that of antioxidant agent, which contributes to the maintenance of biological membrane fluidity by removing free radicals [6]. In the CNS these properties reflect the inhibition of neuronal excitability [39]. The circadian variation in convulsive phenomena [37], which suggests the participation of a biological clock, implies there is a temporal association between the intensity and duration of some types of epileptic seizures and the nocturnal production of aMT. The biological clock is situated, at least in part, in the suprachiasmatic nuclei (SCN) of the hypothalamus, with GABA being the main neurotransmitter. The administration of GABAergic

modulators, including aMT itself and steroids, may modify the phase of some biological rhythms [11,27]. Moreover, increasing GABAergic neurotransmission is one of the most important action mechanisms of antiepileptic drugs, including benzodiazepines (BNZ) and valproic acid, which in turn decreases circulating aMT levels [23,32]. In several experimental conditions it has been shown that aMT administration increases GABA levels in the brain [7,15], modulates the GABA-BNZ receptor complex [12], and modulates membrane permeability by modulating the activity of the Na^+,K^+-ATPase of the brain [2,3]. All these mechanisms confer on aMT a potentially important degree of anticonvulsant activity. In fact, pinealectomy has been associated with tonic-clonic convulsions, which are counteracted in pinealectomized animals after aMT administration [36,41]. Moreover, biochemical pinealectomy, produced by the administration of an anti-aMT antibody, also induces epileptogenic seizures [17]. The anticonvulsant activity of aMT

Table 1 Descriptive statistics of age and seizure duration of both study groups

	Day group (n = 34)		Night group (n = 20)	
	age (months)	seizure duration* (minutes)	age (months)	seizure duration* (minutes)
mean (range)	43.71 (2–168)	10.06 (1–45)	66.35 (9–148)	9.45 (3–20)
standard deviation	51.81	10.64	42.43	4.75

*No differences in seizure duration between groups

was further characterized in a recent model of pentylenetetra-zole-induced epilepsy in rats [10].

The administration of aMT to normal subjects produces an attenuation of the electroencephalographic (EEG) rhythm together with sleep induction [46,48]. An increase in aMT levels after convulsive seizures, whether febrile or epileptic, has also been reported [29]. In pediatric patients, aMT administration induces sleep [21], decreases seizures [30] and myoclonus [20], which is in accordance with the role of aMT as a controller of the motor circuits [14].

Taken together, these data suggest that seizures induce changes in aMT levels, and that aMT administration decreases both the intensity and the duration of seizures. However, the course of aMT production during the hours following the convulsion is unknown. Thus, we consider it worthwhile to examine the levels of aMT up to 24 hours after the convulsion, to determine whether there is any correlation between these changes in aMT and the evolution of the disorder.

Materials and Methods
▼
Patients
Fifty-four children ranging in age from 2 months to 14 years were recruited at the University of Granada Hospital, Andalusia (southern Spain), uniformly distributed throughout the year. Informed consent was obtained from the parents and authorization was received from the hospital's Ethical Committee, in accordance with the Helsinki Declaration. Convulsive seizures were classified in accordance with the WHO's Epilepsy Dictionary and the WHO Commission on Classification and Terminology (1989). The patients were divided into two subgroups: the Day group, comprising 34 patients with the convulsive episode occurring during the daytime (13 febrile convulsions, 21 episodes of epileptic convulsions), sampled between 09.00 and 21.00 h, and the Night group, comprising 20 patients with the convulsive episode occurring during the night (8 febrile convulsions, 12 epileptic convulsions), and sampled between 21.00 and 09.00 h. This division into day and night subgroups was carried out in order to compare the aMT concentrations in these periods, since this hormone presents a circadian rhythm in human plasma with a peak between 02.00 and 04.00 h and a nadir between 14.00 and 16.00 h [24]. The hospital's regular light-dark schedule (lights on from 08.00 to 21.00 h was observed, with an ambient luminance of 300 – 450 lux when the serum samples (one for each patient) were taken. The data obtained for each patient included a complete clinical history, somatometric development and clinical and routine biochemical data, including age, sex, time and duration and type (febrile or epileptic) of seizure. The descriptive statistic of the variables 'age' and 'seizure duration' of both study groups is included in ◉ **Table 1**. For every patient, blood samples were obtained at the time of the seizure (in the patients admitted to Hospital previously to the
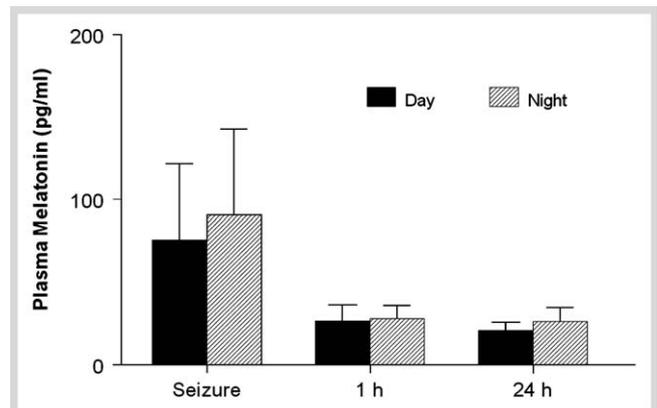

Fig. 1 Day and night aMT levels in serum of patients during the convulsive episode as well as 1 and 24 hours after the seizure.

convulsive episode) immediately after the administration of diazepam, or prior to 5 minutes after the convulsive episode in the case of patients arriving to the Emergency Room Hospital) and 1 h and 24 hours later (±15 minutes in both cases, from insertion of the canula). As our study was based exclusively on patients admitted to Hospital during a convulsive episode, the mean duration of each one of these was prolonged. After 1 h at room temperature, the blood samples were centrifuged and the serum was frozen to −20 °C until the aMT assay was performed.

Melatonin determination
Plasma aMT concentration was measured by a commercial radio-immunoassay (WHB, Bromma, Sweden) as previously described [19]. Intra-assay and inter-assay variations were 11.3 and 16.3 %, respectively. Recovery of aMT was assessed by adding standard aMT, which gave values of 84.4 %. Serum aMT is expressed in pg/mL.

Statistics
All results are expressed as mean ± SD. Statistical analysis of the results included comparison of the data with Bonferroni's test, and one-way ANOVA.

Results
▼
◉ **Fig. 1** shows the aMT values recorded in the study groups (Day and Night groups) at different times after the convulsive period. On the first of these occasions, aMT values were high and similar in both groups (Day group, 75.64 ± 45.91 and Night group, 90.69 ± 51.85 pg/ml). These values had fallen significantly (p < 0.001) one hour later (Day group, 26.33 ± 10.15 and Night group, 27.78 ± 7.82 pg/ml). Although 24 hours later, the aMT values in both groups were somewhat lower than 1 h after the sei-

zures (Day group, 20.68 ± 5.13 and Night group, 26.19 ± 8.59 pg/ml), the difference was not statistically significant.

Discussion
▼

The results show time-dependent changes in aMT levels, associated with seizures. It is suggested that these changes are due to neuronal hyperexcitation and/or seizure activity, since they disappear soon after the convulsive episode. Both our group [30, 31] and others [9] have previously reported that aMT increases after either febrile or epileptic seizures in children. We have also demonstrated the existence of a linear correlation between aMT concentration and the duration of the convulsive episode [28]. The increase in aMT after seizures may constitute an acute response of the body to seizures, inhibiting neuronal excitability [9, 28, 29, 31]. Changes in aMT levels associated with epilepsy have been analyzed in adult patients with intractable temporal lobe epilepsy, with a very low aMT concentration being recorded immediately before the seizure, this increases significantly during the seizure [9]. Thus, the present study was designed to determine the duration of the aMT increase in response to seizures. For this reason, the experimental group comprised patients with a convulsive pathology derived from diverse etiologies. The untreated patients and those given anticonvulsants were randomly and uniformly distributed among the Day and Night groups. The first assay confirmed the aMT increase during the seizures in both the Day and the Night groups, although the pineal response was higher during the night. The assay at one hour after the seizure showed an important decrease in aMT levels in the Day and Night groups, which remained low until 24 hours later. These results suggest that the mechanism(s) that stimulated aMT production at the time of seizures, promptly ceased to have an effect, and thus that aMT may acts as a transient endogenous anticonvulsant mechanism [16]. It should be borne in mind that the endogenous anticonvulsant systems may be primarily (genetically) or secondarily (due to repetitive convulsive episodes) altered in epileptic patients. This transient increase in aMT may also be responsible for the sleepiness that occurs during the refractory postictial period, since aMT has hypnotic properties [47]. The ethical limitations inherent to all research involving pediatric patients prevented us from obtaining a baseline sample (i.e., in the absence of a recent convulsive episode) in the same patients, at the same time; although in this study, to a certain extent each patient may be considered his/her own control (e.g. the melatonin value of the sample obtained at 24 hours after the seizure). For the sake of illustration, let us note that in a previous study by our group, in normal patients (control group) of comparable sex and age (and using the same RIA method), the mean concentration of melatonin was 26.39 ± 9.38 and 53.22 ± 20.87 pg/ml, respectively in the day (09:00–21:00 h) and night groups (21:00–09:00 h) [31]. These results in healthy children reinforce our affirmation about time-dependent changes in aMT levels associated with seizures. Assessing changes in concentrations of melatonin associated with a convulsive episode and determining the potential therapeutic utility of the hormone should, logically, be done on the basis of large, controlled trials. These, moreover, should make it possible to define specific modifications associated with different types of epilepsy. The anticonvulsant properties of aMT have been demonstrated using aMT in the treatment of a child with progressive myoclonic epilepsy. For this clinical assay, 20 mg aMT at 09.00 h and 100 mg aMT at 21.00 h were administered to maintain, albeit at pharmacological level, the circadian rhythm of aMT. With this pattern of treatment, it was possible to obtain clinical control of the seizures, associated with an improvement of the psychomotor activity, with no side effects [30]. Another report further supports the anticonvulsant properties of aMT [18]. However, high doses of aMT, such as 500 mg/day, have been associated with hyperexcitability and generalized myoclonus [30]. Using electroencephalographic recordings, other authors have also found proconvulsive electrical activity in humans after pharmacological doses of aMT [43]. It has been suggested that acute secretory episodes of aMT, such as those produced during the night [33], may cause some proconvulsant activity [43]. Another study has reported generalized seizures in neurologically disabled children after low doses of aMT [44]. However, these results are difficult to analyze due to the multiple alterations showed by the children. Thus, one important point to consider is the threshold dose of aMT for its proconvulsive activity. This dose may vary significantly among different individuals, in a similar way to pyridoxine-dependent seizures. The suggested pro- and anti-convulsive duality of aMT brings to mind the pro- and anti-gonadal effects of the hormone, which also depend on the time of its administration [13, 38].

The cellular mechanisms of the seizures are not totally understood. Since a low activity of cerebral Na^+,K^+-ATPase has been related with several types of human and experimental epilepsy [1, 40, 45], and aMT increases the activity of this enzyme [1], the physiological increase of aMT after seizures may be related to the control of the sodium pump. The anticonvulsant effect of aMT may also involve the reinforcement of the GABAA-BNZ neurotransmission, which induces hyperpolarization [5, 14]. In some epileptic models such as post-traumatic epilepsy, it is suggested that the anticonvulsant activity of aMT may depend, at least in part, on its antioxidant activity [22]. Future, large controlled trials, should define the interrelations between epilepsy type, the parameters of oxidative stress and melatonin concentration.

Besides the hypersecretion of aMT during seizures, studies have reported an alteration in the metabolic pathways of tryptophan, mainly in the kynurenine pathway [8], during seizures. These changes include a shift in the metabolic pathway towards the production of kynurenines with NMDA antagonistic properties such as kynurenic acid during the day, and the production of aMT during the night [34, 35]. Our hypothesis is that during seizures, the deviation of tryptophan metabolism towards hyperproduction of aMT not only elevates the levels of an anticonvulsant, but decreases that of quinolinic acid, a proconvulsant endogenous compound [10, 25, 26].

In summary, a convulsive episode is capable of inducing aMT production at any time in the day. This short-term response puts a high concentration of this neuroprotective compound into circulation [42], which may help to counteract excitability. The amplitude of the pineal response displays a high level of interindividual variability, which should be further analyzed in terms of its anticonvulsant efficiency.

Acknowledgments
▼

References

1 *Acuña-Castroviejo D, Castillo JL, Fernández B, Gomar MD, Del Águila CM.* Modulation by pineal gland of ouabain high-affinity binding sites in rat cerebral cortex. Am J Physiol 1992; 262: R698–R706

2 *Acuña-Castroviejo D, Del Águila CM, Fernández B, Gomar MD, Castillo JL.* Pinealectomy increases ouabain high-affinity binding sites and dissociation constant in rat cerebral cortex. Neurosci Lett 1991; 127: 227–230

3 *Acuña-Castroviejo D, Del Águila CM, Fernández B, Gomar MD, Castillo JL.* Characterization of ouabain high-affinity binding to rat cerebral cortex. Modulation by melatonin. Eur J Pharmacol 1992; 226: 59–67

4 *Acuña-Castroviejo D, Escames Rosa G, Macías M, Muñoz-Hoyos A, Molina-Carballo A, Arauzo M et al.* Cell protective role of melatonin in the brain. J Pineal Res 1995; 19: 57–63

5 *Acuna-Castroviejo D, Lowenstein PR, Rosenstein R, Cardinali DP.* Diurnal variations of benzodiazepine binding in rat cerebral cortex: disruption by pinealectomy. J Pineal Res 1986; 3: 101–109

6 *Acuña-Castroviejo D, Martín M, Macías M, Escames G, León J, Khaldy H et al.* Melatonin, mitochondria, and cellular bioenergetics. J Pineal Res 2001; 30: 65–74

7 *Anton-Tay F.* Melatonin: Effects on brain function. Adv Biochem Psychopharmacol 1974; 11: 315–324

8 *Bauer J, Kaufmann P, Klingmüller D, Elger CE.* Serum prolactin response to repetitive epileptic seizures. J Neurol 1994; 241: 242–245

9 *Bazil CW, Short D, Crispin D, Zheng W.* Patients with intractable epilepsy have low melatonin, which increases following seizures. Neurology 2000; 55: 1746–1748

10 *Bikjdaouene L, Escames G, Leon J, Ferrer JM, Khaldy H, Vives F et al.* Changes in brain amino acids and nitric oxide after melatonin administration in rats with pentylenetetrazole-induced seizures. J Pineal Res 2003; 35: 54–60

11 *Cardinali DP, Golombek DA.* The rhythmic GABAergic system. Neurochem Res 1998; 23: 607–614

12 *Cardinali DP, Lowenstein PR, Rosenstein RE, Gonzalez-Solveyra C, Sarmiento MI, Romeo HE et al.* Functional links between benzodiazepine and GABA receptors and pineal activity. Adv Biochem Psychopharmacol 1986; 42: 155–164

13 *Carter DS, Goldman BD.* Progonadal role of the pineal in the Djungarian hamster (*Phodopus sungorus sungorus*): mediation by melatonin. Endocrinology 1983; 113: 1268–1273

14 *Castillo-Romero JL, Vives-Montero F, Acuna-Castroviejo D.* Paradoxical effects of melatonin on spontaneous neuronal activity in the striatum of sham-operated and pinealectomized rats. J Pineal Res 1992; 12: 149–154

15 *Datta PC, King MG.* Melatonin: Effects on brain and behavior. Neurosci Biobehav Rev 1980; 4: 451–458

16 *Dragunow M.* Endogenous anticonvulsant substances. Neurosci Behav Physiol 1986; 10: 229–244

17 *Fariello RG, Bubenik GA, Brown GM, Grota LJ.* Epileptogenic action of intraventricularly injected anti-melatonin antibody. Neurology 1977; 27: 567–570

18 *Fauteck J, Schmidt H, Lerchl A, Kurlemann G, Wittkowski W.* Melatonin in epilepsy: first results of replacement therapy and first clinical results. Biol Signals Recept 1999; 8: 105–110

19 *Fernández B, Malde JL, Montero A, Acuña D.* Relationship between adenohypophyseal and steroid hormones and variations in serum and urinary melatonin levels during the ovarian cycle, perimenopause and menopause in healthy women. J Steroids Biochem 1990; 33: 257–262

20 *Jan JE, Connolly MB, Hamilton D, Freeman RD, Laudon M.* Melatonin treatment of non-epileptic myoclonus in children. Dev Med Child Neurol 1999; 41: 255–259

21 *Jan MM.* Melatonin for the treatment of handicapped children with severe sleep disorders. Pediatr Neurol 2000; 23: 229–232

22 *Kabuto H, Yokoi I, Ogawa N.* Melatonin inhibits iron-induced epileptic discharges in rats by suppressing peroxidation. Epilepsia 1998; 39: 237–243

23 *Kabuto M, Namura I, Saitoh Y.* Nocturnal enhancement of plasma melatonin could be suppressed by benzodiazepines in humans. Endocrinol Jpn 1986; 33: 405–414

24 *Klein DC.* Photoneural regulation of the mammalian pineal gland. In: Clark S, Evered D, eds. Photoperiodism: melatonin and the pineal. London: Ciba. Found.Symp., 1985; 129–148

25 *Lapin IP, Mirzaev SM, Ryzov IV, Oxenkrug GF.* Anticonvulsant activity of melatonin against seizures induced by quinolinate, kainate, glutamate, NMDA, and pentylenetetrazole in mice. J Pineal Res 1998; 24: 215–218

26 *Manev H, Uz T, Kharlamov A, Joo JY.* Increased brain damage after stroke or excitotoxic seizures in melatonin deficient rats. FASEB J 1996; 10: 1546–1551

27 *Márquez de Prado, Castañeda TR, Galindo A, del Arco A, Segovia G, Reiter RJ et al.* Melatonin disrupts circadian rhythms of glutamate and GABA in the neostriatum of the aware rat: a microdialysis study. J Pineal Res 2000; 29: 209–216

28 *Molina A, Muñoz A, Rodríguez T, Acuña D, Moreno F, Ruiz C.* Interaction between duration of convulsion, refractory postseizure period and melatonin levels. Neuroendocrinology 1990; 52: 102 [Abstract]

29 *Molina-Carballo A, Acuña-Castroviejo D, Rodríguez-Cabezas T, Muñoz-Hoyos A.* Effects of febrile and epileptic convulsions on daily variations in plasma melatonin concentration in children. J Pineal Res 1994; 16: 1–9

30 *Molina-Carballo A, Muñoz-Hoyos A, Reiter RJ, Sánchez Forte M, Moreno Madrid F, Rufo Campos M et al.* Utility of high doses of melatonin as adjunctive anticonvulsant therapy in a child with severe myoclonic epilepsy: Two years' experience. J Pineal Res 1997; 23: 97–105

31 *Molina-Carballo A, Muñoz-Hoyos A, Rodríguez-Cabezas T, Acuña-Castroviejo D.* Day night variations in melatonin secretion by the pineal gland during febrile and epileptic convulsions in children. Psychiat Res 1994; 52: 273–283

32 *Monteleone P, Tortorella A, Borriello R, Natale M, Cassandro P, Maj M.* Suppression of nocturnal plasma melatonin levels by evening administration of sodium valproate in healthy humans. Biol Psychiat 1997; 41: 336–341

33 *Muñoz-Hoyos A, Jaldo R, Molina-Carballo A, Escames G, Macías M, Fernández-García JM et al.* Characterization of nocturnal ultradian rhythms of melatonin in children with growth hormone-dependent and independent growth delay. J Clin Endocrinol Metab 2001; 86: 1181–1187

34 *Muñoz-Hoyos A, Molina-Carballo A, Macías M, Rodríguez-Cabezas T, Martín-Medina E, Narbona-López E et al.* Comparison between tryptophan, methoxyindole and kynurenine metabolic pathways in normal and preterm neonates and in neonates with acute fetal distress. Eur J Endocrinol 1998; 139: 89–95

35 *Muñoz-Hoyos A, Molina-Carballo A, Rodríguez-Cabezas T, Uberos-Fernández J, Ruiz-Cosano C, Acuna-Castroviejo D.* Relationships between methoxyindole and kynurenine pathway metabolites in plasma and urine in children suffering from febrile and epileptic seizures. Clin Endocrinol (Oxf) 1997; 47: 667–677

36 *Philo R.* Catecholamines and pinealectomy convulsions in the gerbil (*Meriones unguiculatus*). In: Reiter RJ, ed., The pineal and its hormones. New York: Alan R. Liss, 1982; 233–241

37 *Quigg M.* Circadian rhythms: interactions with seizures and epilepsy. Epilepsy Res 2000; 42: 43–55

38 *Reiter RJ.* Mechanisms of control of reproductive physiology by the pineal gland and its hormones. In: Reiter RJ, Fraschini F, eds. Advances in Pineal Research 2000. London, John Libbey & Co Ltd., 1987; 109–125

39 *Reiter RJ, Guerrero JM, Escames G, Pappolla MA, Acuña-Castroviejo D.* Prophylactic actions of melatonin in oxidative neurotoxicity. Ann N Y Acad Sci 1997; 825: 70–78

40 *Renkawek K, Renier WO, Pont JJHHM de, Vogels OJM, Gabreëls FJM.* Neonatal status convulsivus, spongiform encephalopathy, and low activity of Na$^+$/K$^+$-ATPase in the brain. Epilepsia 1992; 33: 58–64

41 *Rudeen PK, Philo RC, Symmes SK.* Antiepileptic effects of melatonin in the pinealectomized Mongolian gerbil. Epilepsia 1980; 21: 149–154

42 *Sánchez-Forte M, Moreno-Madrid F, Muñoz-Hoyos A, Molina-Carballo A, Acuña-Castroviejo D, Molina-Font JA.* The effect of melatonin as anticonvulsant and neuronal protector. Rev Neurol 1997; 25: 1229–1234

43 *Sandyk R, Tsagas N, Anninos PA.* Melatonin as a proconvulsive hormone in humans. Int J Neurosci 1992; 63: 125–135

44 *Sheldon SH.* Pro-convulsant effects of oral melatonin in neurologically disabled children. Lancet 1998; 351: 1254

45 *Sugawara T, Tsurubuchi Y, Agarwala KL, Ito M, Fukuma G, Mazaki-Miyazaki E et al.* A missense mutation of the Na+ channel alpha II subunit gene Na(v)12 in a patient with febrile and afebrile seizures causes channel dysfunction. Proc Natl Acad Sci USA 2001; 98: 6384–6389

46 *Wassmer E, Quinn E, Whitehouse W, Seri S.* Melatonin as a sleep inductor for electroencephalogram recordings in children. Clin Neurophysiol 2001; 112: 683–685

47 *Zhdanova IV, Dollins AB, Lynch HJ, Wurtman RJ.* Induced melatonin levels comparable to normal nocturnal levels affect human sleep. Clin Res 1994; 42: A260

48 *Zhdanova IV, Lynch HJ, Wurtman RJ.* Melatonin: a sleep-promoting hormone. Sleep 1997; 20: 899–907