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Circadian effects of dopaminergic treatment in restless legs syndrome

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Abstract

Background and purpose: Although an essential diagnostic feature of restless legs syndrome (RLS) is the presence of circadian symptom variations, with an increase in the evening or at night, the mechanisms underlying this time-bound variation remain unknown. Since dopaminergic mechanisms seem to play a central role in the pathophysiology of RLS, it is likely that circadian variations in the dopaminergic system or factors affecting it cause the nightly increase. The reverse is also possible; dopaminergic medication might affect melatonin function, a key element of the circadian system. The present study investigated the effects of dopaminergic medication on melatonin secretion in RLS.

Patients and methods: Eight previously untreated patients diagnosed with idiopathic RLS underwent a three-week, open-labeled treatment with 400 mg L-DOPA (+100 mg CarbiDOPA). Dim Light Melatonin Onset (DLMO), a marker of circadian phase, was determined before and after treatment.

Results: Compared to baseline, earlier DLMO was found in L-DOPA treated patients (21:00 ± 1:20 vs. 18:50 ± 0:55; $P < 0.05$). Anticipation of DLMO was more marked in the subgroup of patients showing augmentation. A positive correlation was observed between change of DLMO, sleep latency and time of onset of symptoms following treatment with L-DOPA.

Conclusions: Our results suggest that L-DOPA may exert chronobiotic effects in RLS.

Keywords: Restless legs syndrome; Dopamine; Circadian system; Dim light melatonin onset; Augmentation; Chronobiotic effects

1. Circadian rhythm of symptoms in RLS

Restless Legs Syndrome (RLS) is a movement disorder that affects up to 5–10% of the general population [1,2]. According to a consensus established by the International Restless Legs Study Group [3], an exacerbation of symptoms in the evening or at night is one of the clinical features that defines the syndrome.

The presence of a circadian variation of symptoms can be biased by the fact that RLS symptoms exacerbate during inactivity, which typically occurs in the evening and at night. Nevertheless, a circadian variation of symptoms has been shown in two studies, specifically addressing the question of a 'true' circadian modulation of symptoms, in which the circadian rhythm of body temperature as well as the 24-h pattern of sensorial and motor symptoms were investigated in RLS patients who underwent sleep deprivation [4,5]. The monitoring conditions in some aspects resembled the methods employed in a constant routine paradigm [6]; patients were asked to keep their activity at a constant, low level and underwent an immobilization test (SIT) [7] at periodic intervals. In one study [5] patients were asked to remain quiet and periodically report their subjective sensations; in the second [4], the SIT was modified to allow subjects to make voluntary movements when they experienced RLS symptoms (modified SIT or mSIT). The studies concluded that the circadian rhythm of body temperature, a marker of circadian phase, does not differ between patients and retrospectively compared healthy subjects. Furthermore, the circadian oscillation of motor and sensorial symptoms was observed even under conditions of sleep deprivation. Thus, waking leg discomfort, motor restlessness, and periodic leg movements during wake (PLMW) induced by the SIT showed a peak in the early portion of the sleep period (23:00–4:00) and a nadir during the early portion of the wake period (9:00–2:00). The period of maximal

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frequency of PLMW coincided with the falling portion of the core temperature cycle. Similarly, periodic leg movements during sleep (PLMS) peaked in the same time period. In addition, sleep deprivation exacerbated the severity of RLS symptoms, suggesting a 'homeostatic drive' as an additional factor modulating RLS symptoms. In summary, these studies suggested that RLS severity was determined not just by activity, but by a circadian factor.

2. Loss of circadian fluctuation

Subjects with severe RLS may have symptoms throughout 24 h, and thus apparently not show circadian variation. When asked, these patients recall having had an evening/night exacerbation of symptoms early in the course of the disease when the symptoms were milder [8]. Similarly, patients with mild RLS, although they may not be aware of worsening symptoms in the evening or night, report that prolonged inactivity more frequently exacerbates symptoms if it takes place in the evening or at night [3].

Another clinical situation during which the circadian variation of symptoms might be lost is severe augmentation, a complication that usually takes place following long term dopaminergic treatment. Augmentation has been reported to occur more commonly with L-DOPA [3,9–11], and involves an earlier onset of symptoms in the day, an increased severity of symptoms, a reduced time at rest before symptoms start, and an expansion of symptoms to the upper limbs and trunk. When augmentation is severe, symptoms appear earlier in the day and can be present around the clock, with an apparent loss of circadian rhythm; there is an overall increase in the severity of RLS symptoms, probably as a result of changes in sensitivity of the dopaminergic system to long term treatment with L-DOPA [9].

3. Dopaminergic function in RLS

There is increasing evidence that suggests a central role of the dopaminergic system in the pathophysiology of RLS [12]. The strongest support is based on the therapeutic effect of dopaminergic drugs [11,13], as well as on the increase of symptoms caused by dopamine receptor blockers [14,15]. In addition, both single photon emission computed tomography (SPECT) and positron emission tomography (PET) studies have detected decreases in D2-receptors in the basal ganglia [16]. However, the reported abnormalities have been mild at most, or even absent in some studies [17]. Further, cerebrospinal fluid dopamine metabolites do not differ between patients and controls [18]. Moreover, analysis of genetic association has not shown any linkage between dopaminergic transmission and RLS [19]. However, most of the previous studies have been performed in the morning or at times at which patients were not symptomatic.

4. Circadian variations in dopaminergic activity

Although an involvement of the dopaminergic system in the pathophysiology of RLS seems undisputed, it does not itself explain the fact that symptoms occur in a time-bound fashion, starting or increasing in the evening and at night. Thus, an independent 'circadian factor' affecting or modulating dopaminergic activity has been suggested.

Both animal [20,21] and human studies [22,23] suggest the existence of circadian variations in dopaminergic activity [24–27]. Circadian variations have been described for key elements in dopaminergic function [28], such as the dopamine transporter, tyrosine hydroxylase and dopamine D1- and D2-receptors. Human data show a distinct circadian variation, with a pattern characterized by an increase in the morning and a nadir in the late evening/night [29,30].

Alternatively, the circadian pattern may not be generated by the dopaminergic system itself but by other factors that indirectly modulate it, such as tetrahydrobiopterin (TH-biopterin), a cofactor of tryptophan and tyrosine hydroxylase [31]. TH-biopterin's brain content shows a circadian oscillation with a daytime increase that parallels dopamine production [21,32]. So far, cerebrospinal fluid (CSF) levels of TH-biopterins have been found to be normal in RLS when collected in the morning [18]. The results remain inconclusive, as no similar samples have been taken while patients were symptomatic at night.

An alternative candidate would be iron, which is also a cofactor of tyrosine-hydroxylase [31] and is low in the CSF of RLS patients [33]. Serum iron shows a marked circadian variation, with a low point in the evening and early night [34], coinciding with maximal severity of symptoms.

5. Neuroendocrine challenges

Neuroendocrine responses to dopaminergic drugs provide an additional perspective to investigate the function of the DA system. As preliminary data have recently shown, nighttime administration of 200 mg L-DOPA caused an increased release of growth hormone (GH) and a reduced secretion of PRL in comparison to morning administration or to controls [35,36]. A significant correlation could be seen between the PLMS-index (number of PLMS per hour of sleep) and the degree of inhibition of prolactin (PRL). If confirmed, these results would support the presence of hypersensitive dopamine (DA) post-synaptic receptors at night. Thus, RLS patients might have increased amplitude of the circadian variation of dopaminergic function when compared to healthy controls.

6. Melatonin and the dopaminergic system

Melatonin is produced in the pineal gland and is secreted at night into the cerebrospinal fluid and blood circulation. In continuous darkness, melatonin rhythms persist with an

suprachiasmatic nuclei (SCN)-driven periodicity. Light–dark cycles synchronize the rhythm and acute light exposure at night rapidly stops melatonin production [37]. The variations in melatonin levels provide the organism with information on the timing and duration of the dark period, regardless of whether the animal is nocturnally or diurnally active [38]. Melatonin acts as a time cue to entrain or phase shift the endogenous clock and, in addition, exerts direct effects on SCN firing rate and gene expression [39]. As a signal to the organism of darkness, melatonin has a major role in regulation of sleep [40]. Along with its sleep inducing properties [41], melatonin increases peripheral vasodilation in humans and reduces body temperature [42].

Melatonin's inhibition of dopamine release has been demonstrated in specific areas of the mammalian central nervous system (hypothalamus, hippocampus, medulla-pons, and retina) [43–45]. Administration of melatonin for 9 weeks to Syrian hamsters caused a progressive decline to 50% in dopamine content of the posterior pituitary [46], suggesting a decrease in tubero-infundibular dopaminergic activity. Furthermore, elevations in tyrosine-hydroxylase in the mediobasal hypothalamus have been found [47]. Melatonin may also exert antidopaminergic effects in the striatum [48].

In humans, evidence for the importance of dopaminergic transmission in the circadian system is still scarce. Prolonged inhibition of pre-synaptic catecholamine synthesis via alpha-methyl-*para*-tyrosine attenuates the circadian rhythm of thyroid stimulating hormone (TSH) secretion [49]. In the SCN melatonin may inhibit dopamine release and perhaps post-synaptic N-methyl-d-Aspartate (NMDA) receptor-mediated responses to the light signal from the retina, because the SCN modulates the sensitivity of dopaminergic neurons to melatonin [48]. Thus, melatonin and dopamine act as mutually inhibitory signals for day and night, respectively. The dopamine system may participate in the entrainment of the biological clock by non-photic cues, including entrainment by melatonin, as well as in the fine-tuning of motor coordination in the striatum [48]. Melatonin may have some clinical use in disorders where the dopaminergic system plays a major role, and potential neuroprotective effects are under discussion [50–52]. Furthermore, preliminary evidence has shown melatonin to be an effective treatment for tardive dyskinesia, a movement disorder caused by long-term dopaminergic blockage of dopaminergic receptors by antipsychotic medication [53]. Interestingly, a recent open-label study showed a therapeutic effect of 3 mg melatonin in nine patients diagnosed with periodic leg movement disorder (without RLS) [54].

7. Effects of dopaminergic treatment on circadian function

As previously stated, long-term treatment with dopaminergic drugs, particularly L-DOPA, can lead to augmentation in 50–85% of cases. The cardinal clinical characteristic of augmentation is the onset of symptoms earlier in the day [3], a feature that could reflect a phase advance of circadian pacemakers. Thus, we investigated under open conditions whether dopaminergic treatment of RLS exerts any effects on DLMO, considered to be one of the most stable phase markers of the endocenous circadian rhythm [55–57]. We further investigated whether these changes differ between patients with and without augmentation.

8. Methods

8.1. Subjects

Eight patients (mean age (SD): 53 years (9.1); 5 men/3 women) participated in the study. All patients met criteria for idiopathic RLS, established by the International Restless Legs Syndrome Study Group [58]. The diagnostic process consisted of a medical history that included a comprehensive interview for sleep disorders, along with a physical and neurological exam. A polysomnographic study (PSG) and a suggested immobilization test were performed in all patients as confirmatory tests for RLS [7]. A blood count and blood chemistry that included serum levels for creatinine, iron, ferritin, and transferrin, were performed along with a determination of urinary creatinine clearance. None of the patients had been treated previously with dopaminergic medication. Any medication with central nervous system (CNS) effects was discontinued at least two weeks prior to the study.

Patients were treated for an average of 21 days (SD ± 5.2) with a fixed schedule of L-DOPA. The oral daily dosage during the first three days of the study was 200 mg (+50 mg carbi-DOPA), followed on the following days by a daily dosage of 400 mg (+100 mg carbi-DOPA). Medication was always administered at 21:00.

8.1.1. Measurement of salivary melatonin

Patients underwent studies for determination of DLMO one day before the beginning of the treatment period with L-DOPA (untreated condition) and at the end of the treatment period (treated condition). On both occasions patients were admitted to the hospital at 15:00 and were told to remain in a room under dim light (<5 lux), as quiet as possible, until 03:00. During that period patients remained seated, except for short walks in order to alleviate symptoms. A snack was offered to patients at 18:00, and a regular dinner at 21:00. No coffee or tea was allowed during the study.

Five milliliter samples of saliva were obtained every hour between 17:00 and 03:00 to determine salivary levels of melatonin. Samples for melatonin were assayed by radio-immunoassay using an antiserum raised in rabbit and an iodinated radiolabel [59]. The sensitivity of the assay was 1.5 pg/ml, and interassay coefficients of variation over the range 10–120 pg/ml ranged from 3.8 to 6.4%. Following

the measurement of salivary melatonin, lights were turned off and a sleep study was performed between 03:00 and 10:00 [60]. DLMO was defined as the time of interception of a sigmoid-shaped curve (fitted through the hourly melatonin concentration) with a criterion-level of 10 pg/ml [55–57].

Patients were rated at baseline and after treatment by means of the International RLS Study Group Rating Scale (IRLS) [61]. Patients also completed, each day during the entire study, a 24-h restless legs agenda on which the severity of symptoms was estimated every hour on a 0–4 scale (0: no symptoms; 4: very severe symptoms). The time of onset of symptoms was calculated for each patient as the mean time of onset of symptoms for the given week. Based on the clinical definition [3], the treating physician decided at the end of treatment which patients had shown symptoms of augmentation. Clinical classification into augmentation (A-RLS) and no augmentation (NA-RLS) was performed blind to the melatonin results.

8.2. Statistical analysis

Due to the small sample size, all statistical analyses were performed by means of non-parametric tests. Comparisons between independent groups were analyzed by means of Mann–Whitney test, and Wilcoxon tests were used for paired analyses (pre-treatment vs. post-treatment). Extreme values that might affect results were studied on all variables.

This exploratory pilot investigation was designed to obtain preliminary evidence prior to the study of a larger number of patients under controlled conditions.

The study was approved by the local Ethics Committee and a written informed consent was obtained from all patients.

9. Results

Eight patients participated in the study (mean (± SD) age 53 years (± 9,1); 5 men/3 women). The estimated mean (± SD) duration of illness was 26 years (± 12,2).

9.1. Treated vs. untreated patients

Fig. 1 shows the profiles of melatonin secretion across time for untreated and treated patients. Treatment with L-DOPA was associated with higher levels of salivary melatonin at 20:00, 21:00 and 22:00. Mean time for DLMO in untreated patients was 21:00 ± 1:20, in contrast to 18:50 ± 0:55 ($P < 0.05$) for treated patients, suggesting an advance of the time of DLMO under treatment with L-DOPA.

Table 1 shows the main clinical and PSG features of patients before and after treatment. As expected, treatment with L-DOPA was associated with a decrease in IRLS score ($P > 0.01$). Furthermore, treated patients had a lower PLMI index ($P < 0.01$), periodic leg movements index (PLMI)

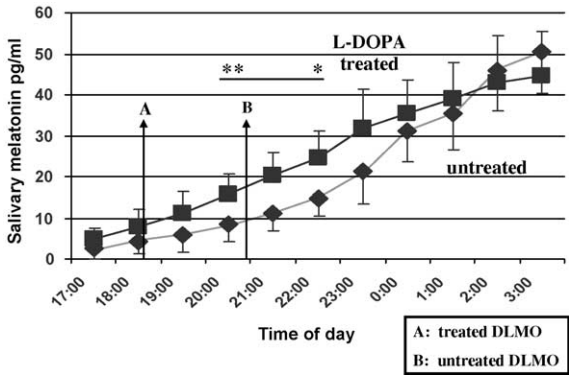

Fig. 1. Salivary levels of melatonin (mean ± SD) across time and treatment conditions in RLS patients ($n = 8$). The arrows show the time of DLMO for each treatment condition. *$P < 0.05$.

($P < 0.01$), sleep latency ($P < 0.05$), and percentage of Stage 1 Sleep ($P < 0.01$), as well as a higher Total Sleep Time ($P < 0.05$), sleep efficiency ($P < 0.01$), and percentage of Slow Wave Sleep ($P < 0.05$). Change across treatment conditions in DLMO was associated with change in sleep latency ($r = 0.703$; $P < 0.05$), as well as with change in time of onset of symptoms (Fig. 2). No other correlation was seen between changes in DLMO and any other clinical or PSG variables.

9.2. Augmentation vs. non-augmentation

Patients were classified as those experiencing signs of augmentation (A-RLS; $n = 4$), and those who did not (NA-RLS; $n = 4$). All patients classified as A-RLS were experiencing at least the cardinal symptom of augmentation (earlier onset of symptoms to the afternoon) [3,9].

Melatonin salivary levels at baseline did not differ between untreated A-RLS and NA-RLS (Fig. 3). Following treatment, A-RLS showed higher melatonin levels at 18:00, 19:00, 20:00, 24:00 and 01:00. Comparison of melatonin salivary levels in A-RLS across treatment conditions showed higher levels in the L-DOPA treated group between 17:00 and 24:00 ($P = 0.06$ at all time points). In the N-RLS group, difference across treatment conditions reached marginal statistical significance exclusively at 21:00, with higher salivary levels for the treated group ($P < 0.7$) (Fig. 3, bottom).

DLMO values did not differ between untreated A-RLS and NA-RLS. While treatment with L-DOPA caused an anticipation of DLMO in A-RLS ($P < 0.6$), no changes were seen in NA-RLS. Following treatment, A-RLS had an earlier DLMO than NA-RLS ($P < 0.05$) (Fig. 3, top).

10. Discussion

Our study shows under open label conditions that a three-week treatment period with 400 mg L-DOPA causes

Table 1
Main clinical and polysomnographic features for the entire group of RLS patients ($n = 8$), before and after treatment with L-DOPA

	Untreated			L-DOPA treated			Untreated vs. treated
	Augmented	Not augmented	*P*	Augmented	Not augmented	*P*	*P*
DLMO (time of day)	21.75 (+0.95)	20.25 (+1.5)	n.s.	18 (+0)	19.75 (+0.5)	*	*
IRLS	27.5 (+3.51)	21 (+4.08)	*	11.5 (+2.08)	10.25 (+2.63)	n.s.	**
PLM-I (PLMs/h)	40.5 (+10.1)	23.5 (+6.85)	n.s.	17.5 (+7.59)	8.75 (+4.34)	*	**
PLM-I arousal	17 (+6.05)	8.25 (+4.57)	n.s.	10 (+4.96)	3.75 (+0.96)	n.s.	*
SL (min)	80 (+58.75)	40.5 (+4.43)	*	31.75 (+3.68)	30 (+10.29)	n.s.	*
WASO (min)	31.5 (+18.06)	42.5 (+11.67)	n.s.	35.25 (+15.28)	25.5 (+9.88)	n.s.	n.s.
TST (min)	306.5 (+75.27)	335 (+14.62)	n.s.	351 (+12.83)	362.5 (+5.80)	n.s.	**
TRT (min)	405 (+14.58)	409.75 (+9.91)	n.s.	396.75 (+12.23)	395.5 (+17.33)	n.s.	n.s.
SE (%)	76.10 (+20.33)	81.83 (+5.02)	n.s.	88.49 (+2.78)	91.83 (+5.43)	n.s.	**
St1 (%)	21.75 (+6.07)	18.5 (+10.14)	n.s.	12 (+1.82)	8.75 (+3.59)	n.s.	**
St2 (%)	59 (+4.32)	67.75 (+2.21)	*	60.5 (+5.26)	57 (+5.35)	n.s.	n.s.
SWS (%)	9 (+6.37)	3.75 (+4.78)	n.s.	12.75 (+3.77)	13.75 (+1.26)	n.s.	*
REM (%)	10.25 (+6.89)	10 (+6.78)	n.s.	14.75 (+8.22)	20.5 (+4.43)	n.s.	(*)

(*) $P < 0.1$; *$P < 0.05$; **$P < 0.01$.

anticipation in DLMO in RLS patients. Furthermore, change in DLMO across treatment conditions was positively correlated with change in sleep latency, as L-DOPA treated patients with earlier times of DLMO were also those with shorter sleep latencies.

The study did not include normal controls. A retrospective comparison between DLMO values in untreated RLS and those published in the literature on healthy subjects is difficult due to methodological differences between studies. Nevertheless, the mean DLMO value in untreated RLS patients is similar to those published for healthy subjects [56,60]. Similarly, the circadian rhythm of body temperature is not likely to be abnormal in untreated RLS [4,5].

Several methodological problems should be mentioned. First, the study included a small sample of patients, and L-DOPA was not administered under controlled conditions. Second, although laboratory and light exposure conditions were controlled during the DLMO study, there was no strict control for body activity, as movement was determined by the severity of symptoms. Furthermore, no control of possible entrainment factors was performed before the beginning of the study. Third, as no dim light melatonin offset (DLMOff) was determined, our results cannot differentiate between a phase advance of the circadian rhythm of salivary melatonin and a retrograde (and perhaps overall) expansion of the shape of its circadian curve. These shortcomings might certainly limit the strength of any conclusions, and our results should be understood as reflecting a pilot, exploratory investigation.

With these methodological limitations in mind, the data suggest that dopaminergic treatment might exert chronobiotic effects in RLS patients, in addition to a reduction of RLS symptoms. Thus, L-DOPA might induce a phase advance of the sleep–wake cycle and thus contribute to the improved sleep seen in RLS patients. A similar phase advance of DLMO has been observed in Parkinson's disease during treatment with L-DOPA [62,63].

Furthermore, the anticipation in the time of DLMO was more pronounced in patients experiencing augmentation than in those who did not. While no differences were seen before treatment between A-RLS and NA-RLS, treatment with L-DOPA was associated with an earlier DLMO, mainly in A-RLS, suggesting that this subgroup contributed most to the anticipation in DLMO observed in the entire group of L-DOPA treated RLS patients. Taken together, the results suggest an earlier DLMO in A-RLS patients. Since the main feature of augmentation is an earlier time of onset of symptoms, several interpretations seem possible. The earlier DLMO might be an indirect reflection of changes in dopaminergic activity during augmentation. Alternatively, it is tempting to speculate a causal implication of the phase advance in DLMO in the pathophysiology of augmentation; L-DOPA might directly or indirectly anticipate the onset of melatonin secretion and indirectly cause changes in the circadian rhythm of body temperature. As subjective symptoms of both RLS and PLMS are known to show

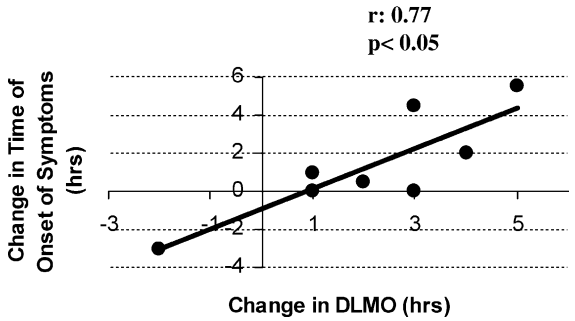

Fig. 2. Correlation between change in DLMO and change in time of onset of symptoms ($n = 8$). Change is calculated as the difference between pre-treatment and L-DOPA-treatment values.

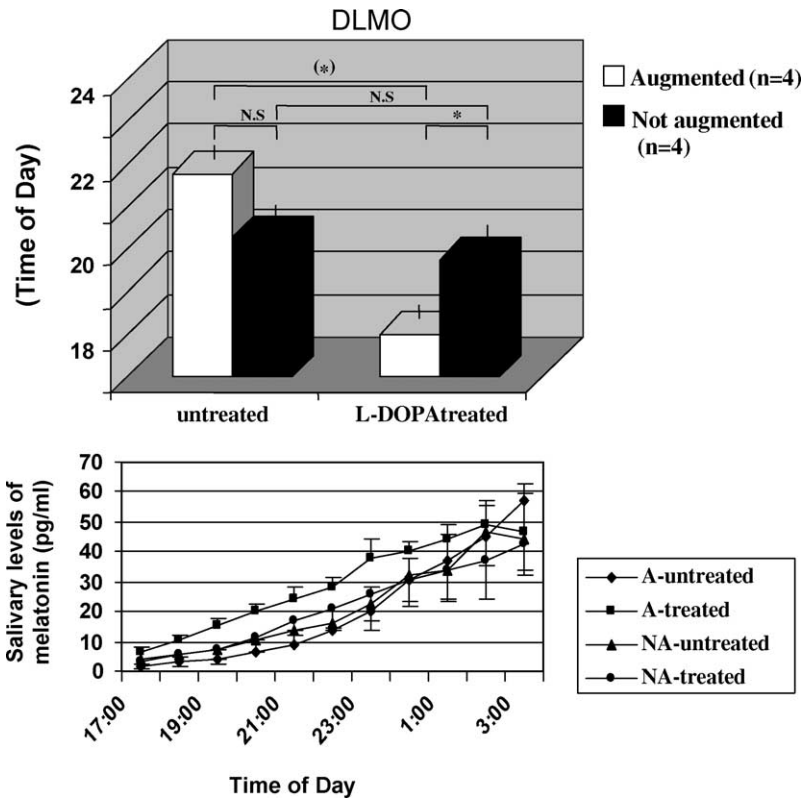

Fig. 3. Top: DLMO values across treatment conditions in RLS patients experiencing augmentation (A-RLS; $n = 4$) and RLS patients without criteria for augmentation (NA-RLS; $n = 4$). (*) $P < 0.1$; *$P < 0.05$. Below: Salivary levels of melatonin (mean ± SD) across time and treatment conditions for both groups of RLS patients (A-RLS and NA-RLS).

maximal severity during the falling phase of the circadian phase of body temperature [4,5], an anticipation of the falling phase of body temperature would ultimately lead to an earlier onset of RLS symptoms in the afternoon or evening, thus partly explaining the symptoms of augmentation.

Should these results be confirmed by larger, controlled studies, they could offer a biological marker that would be of interest for clinical studies of augmentation. Our study also suggests chronobiotic effects of L-DOPA that could be helpful in understanding its mechanism of action and its effects in RLS as well as in other movement disorders.

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