Adrenergic reactions during N3 sleep arousals in sleepwalking and sleep terrors: The chicken or the egg?

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Adrenergic reactions during N3 sleep interruptions in arousal disorders: The chicken or the egg?

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SUMMARY

To understand the mechanisms of N3 sleep interruptions in patients with sleepwalking episodes and/or sleep terrors (SW/ST), we evaluated whether autonomic reactions preceded or accompanied behavioural arousals from NREM sleep stage N3. In 20 adult patients with SW/ST and 20 matched controls without parasomnia, heart rate and pulse wave amplitude were measured beat-to-beat during the 10 beats preceding and during the 15 beats succeeding a motor arousal from N3 sleep. Respiratory rate and amplitude were measured during the same 25 successive beats. In patients with SW/ST, the N3 arousals were associated with a 33% increase in heart rate, a 57% decrease in pulse wave amplitude (indicating a major vasoconstriction), a 24% increase in respiratory rate and a doubling of respiratory amplitude. Notably, tachycardia and vasoconstriction started 4 seconds before motor arousals. A similar profile (tachycardia and vasoconstriction gradually increasing from the 4 s preceding arousal, post-arousal increase of respiratory amplitude, but no polypnea) was also observed, with a lower amplitude, during the less frequent 38 quiet N3 arousals in control subjects. Parasomniac arousals were associated with greater tachycardia, vasoconstriction and polypnea than quiet arousals, with the same pre-arousal gradual increases in heart rate and vasoconstriction.

Autonomic arousal occurs 4 seconds before motor arousal from N3 sleep in patients with SW/ST (with a higher adrenergic reaction than in controls), suggesting that an alarming event during sleep (possibly a worrying sleep mentation or a local, deep arousal) causes the motor arousal.

INTRODUCTION

Patients with sleepwalking episodes or sleep terrors (SW/ST) exhibit sudden abnormal behaviours arising from sleep, mostly from N3 sleep (American Academy of Sleep Medicine, 2014). Patients usually open their eyes, look around with a confused gaze, sit, stand, walk, talk, scream or flee their bed (Derry *et al.*, 2009). There is a continuum between the different behavioural patterns of arousal parasomnias. Specifically, the behavioural patterns of NREM parasomnias appear with a hierarchical order, with arousal behaviours being the fundamental component, possibly followed by abnormal agitated conducts which can be further accompanied by distressed emotional manifestations (Derry *et al.*, 2009). Several clinical, polygraphical and brain functional imaging studies support the concept that these behaviours occur during local (primarily thalamo-amygdalo-cingulo-cortical) arousals from N3 sleep, disengaged from the control of the prefrontal and frontal associative cortex (Bassetti *et al.*, 2000; Terzaghi *et al.*, 2009; Terzaghi *et al.*, 2012; Flamand *et al.*, 2018). The mental content is poor, often amnestic, but adult patients occasionally report a brief visual scene, containing imminent danger or misfortune (Schenck *et al.*, 1989; Oudiette *et al.*, 2009; Zadra *et al.*, 2013) and sometimes longer scenarios (Uguccioni *et al.*, 2013). Regular episodes of SW/ST persists in 2-4% of adults (Ohayon *et al.*, 1999; Ohayon *et al.*, 2012) and leads to a high risk of self-induced injury or violence to other persons, disturbed sleep, fatigue, excessive daytime sleepiness, shame, anxiety and altered quality of life (Lopez *et al.*, 2013; Zadra *et al.*, 2013; Arnulf *et al.*, 2014; Carrillo-Solano *et al.*, 2016).

The cause of SW/ST remains unknown, although familial vulnerability and factors that increase sleep depth (e.g., sleep debt or fever) and that cause arousals during N3 sleep (e.g., noise or contact) promote abnormal behaviours (Pressman, 2007). However, most N3 arousals appear to be spontaneous in SW/ST, including no external triggers, and no change preceding the arousal whether in the surface EEG (Schenck *et al.*, 1998; Gaudreau *et al.*, 2000; Pilon *et al.*, 2006; Jaar *et al.*, 2010) or stereo EEG (Terzaghi *et al.*, 2012). Nevertheless, rare hypersynchronous delta waves (Guilleminault *et al.*, 2001) and relatively short-duration increases in slow wave oscillations in the two minutes preceding behavioural (but not quiet) N3 interruptions have been found in patients with arousal disorders (Espa *et al.*, 2000). In addition, recent EEG tools allowed to observe more gradual and local changes in the brain activity, within up to 20 sec before a confusional arousal. The activity of the primary motor cortex and cingulate cortex increased 5 sec before the onset of confusional arousal in stereo EEG in an adult patient (Terzaghi *et al.*, 2009). There was an “arousing” (increased beta band) brain activation in the cingulate motor area during the 4 sec preceding the N3 interruption, using current density EEG in 20 adults with arousal disorders (Januszko *et al.*, 2016). Changes in EEG functional connectivity were also observed in the 20 sec preceding a parasomniac event (compared to 2 min before the event), suggesting that both arousing (increased functional connectivity in the alpha and beta bands over a wide anteroposterior network) and sleep (increased delta and theta power) processes coexisted before the parasomniac behaviour (Desjardins *et al.*, 2017). In 19 confusional arousals monitored with stereo EEG, Flamand et al. (2018) identified an increase in delta activity, predominantly in the frontal regions, in the last few seconds before behaviour onset.

When looking at cardiac and respiratory activity surrounding ST episodes in adults, Fisher et al. observed that the episodes of tachycardia typically arose from "physiological quiescence, with no gradual build-up" (Fisher *et al.*, 1973). ST episodes but also SW episodes and confusional arousals are commonly associated with increased sympathetic arousal (Schenck *et al.*, 1998). However, it was unclear whether autonomic arousal preceded or accompanied the behavioural episode. The heart rate increased one heart beat interval before the phases of transitory activation in N3 (phases which included EEG changes, eye movements, increased respiration or EMG activation) in 10 sleepwalkers (Busek *et al.*, 2005). Therefore, we aimed at determining more precisely whether the autonomic arousal preceded (the "egg") or accompanied (the "chicken") the behavioural episode. For this purpose, we measured the profile of heart rate changes, prior to and during N3 arousals in patients with SW/ST and in controls. In addition, as the respiratory reaction and vascular tone changes had not been quantified in these three previous studies, we also measured the profile of respiratory rate and amplitude changes, as well as vascular tone, prior and during the pathological and non-pathological arousals in N3 sleep.

METHODS

Participants

We recruited 20 consecutive patients referred to the Sleep Disorder Unit for SW, ST, or both. They met the general diagnostic criteria for disorders of arousal (American Academy of Sleep Medicine, 2014), which include (i) recurrent episodes of incomplete awakening from sleep, (ii) inappropriate or absent responsiveness to

efforts of others to intervene or redirect the person during the episode, (iii) limited or no associated cognition or dream imagery, (iv) partial or complete amnesia for the episode and (iv) the disturbance was not better explained by another sleep disorder, mental disorder, medical condition, medication, or substance use. In addition, SW was defined as (i) a history of ambulation during sleep, and (ii) the persistence of sleep or impaired judgment during ambulation. Sleep terror was defined as (i) a history of sudden episodes of terror occurring during sleep, usually initiated by a cry or loud scream, with sympathetic and behavioural manifestations of intense fear; and (ii) difficulty in arousing the person, mental confusion when awakened from an episode, complete or partial amnesia of the episode, or dangerous behaviours. Additional inclusion criteria were (i) at least one motor episode occurring during N3 sleep, and (ii) no treatment for more than one month. The control group comprised 20 healthy subjects who had visited the sleep disorder unit to take part in various trials. Only healthy subjects with at least one awakening episode during N3 sleep without any treatment for more than 1 month and without any sleep disorders were included. These subjects were matched for age and sex with the SW/ST patients. The patients gave their written consent to take part in the study, which was approved by the ethics committee Ile de France-06 as part of a larger study on hypnosis in sleepwalking (NCT02648568 on clinicaltrial.gov). The healthy subjects also signed a consent (depending on the study they were control for, studies which had been approved by the ethics committee Ile de France-06), which allowed the investigators to reuse their data in other researches.

Evaluations

Participants had a medical interview with a sleep specialist and completed the Epworth sleepiness scale (Johns, 1991), and the Paris Arousal Disorder Severity Score (Arnulf *et al.*, 2014). Patients underwent a videopolysomnography during two consecutive nights, whereas controls underwent a single night. Only the first night was used for this study. The videopolysomnography included recordings from 9 EEG leads (Fp1, C3, T3, O1, Fp2, C4, T4, O2, and A2), two bilateral electro-oculograms, surface EMG electrodes of the *mentalis* and right and left *anterior tibialis* muscles, nasal pressure, tracheal sounds, chest and abdomen movements measured via plethysmography, electrocardiogram (EKG), pulse oximeter and infrared video and audio (Medatec Ltd, France). The sleep stages and the motor and respiratory events were scored by experienced neurologists following standard methods (Iber *et al.*, 2007).

N3 arousals were defined as “any increase in EMG on the chin channel, which is accompanied by a change in pattern on any additional channel” (Rechstchaffen and Kales, 1968) lasting for more than 3 s. The classical definition of arousal (any increase in EEG rhythm lasting more than 3 s) could not be used because it did not fit with the N3 arousals, which may combine an apparently awake behaviour and a hypersynchronous, desynchronized or mixed (on the same or on different channels) EEG rhythm. For the same reasons, the exact time of the N3 arousal was set at the precise moment of sudden chin muscle tone enhancement (30% greater than stable, previous muscle tone). The associated behaviour was analyzed on the video/audio clip. If there were no visible movements or quiet, routine movements (scratching the nose, touching the nasal sensor, repositioning oneself, all behaviours performed with closed eyes), the episode was qualified as a “quiet arousal”. If the motor behaviour was sudden, including an immediate opening of the eyes, staring or looking around,

raising head or torso, any facial or vocalizations expression of fear or surprise, sitting in bed, standing, or screaming, the episode was qualified as “parasomniac arousal”. Although not formally measured, the duration of quiet arousals was similar in patients and in healthy controls and around 15 s, with increased chin muscle activity at the beginning of the arousal in both cases.

The autonomic (heart rate, respiration and vessel tone) changes concomitant to each N3 arousal were measured as indicated in Figure 1. The instantaneous heart rate was automatically calculated by the Compumedics Profusion Software, calculation which is based on the pulse wave signal from the oximetry and not on the EKG. The absence of artefactual measures was checked via manual measure of the EKG signal. Pulse oximetry wave amplitude was manually measured, wave by wave, as the minimum to maximum of each wave of the percutaneous pulse signal. This signal is automatically calibrated by the computer and is used here as a relative measure (prior to and during the arousal), proper to each subject and each episode of arousal. This is a finger plethysmography signal, which can be considered as a surrogate measure of blood pressure. A decrease in pulse wave amplitude indicates a vasoconstriction. Only arousals with no concomitant movement of the finger (as check on the concomitant video) were kept in the analysis, to ensure that the signal changes were not artefacts of movement. Heart rate and pulse wave amplitude were measured beat to beat on a segment comprising 10 beats before the episode and 15 beats after the onset of the episode. Typically, an increase in heart rate (tachycardia) and a decrease in pulse wave amplitude (vasoconstriction) indicate a sympathetic reaction (Shelley *et al.*, 1997). The respiratory rate (peak to peak measure of the signal on the thoracic belt) and amplitude (in mL) were measured based on the thoracic plethysmography signal during the 3 respiratory cycles preceding the

episode, during the cycle when the episode started (the episode could start during inspiration, expiration or post-expiration pause), and during 5 cycles after the initiation of the episode. This duration was chosen to evaluate the stability of the autonomic system prior to awakening and its changes during the early onset of awakening. We did not extend the analysis after 15 s post-arousal because the behaviour evolution was variable, with some subjects resuming sleep while others evolving toward complete awakening. Because the measures were manually performed, artifacts (e.g., contamination of the EKG signal by myographic signals, contamination of all signals by movements, etc.) were carefully excluded. In addition, any arousal secondary to a noise in the room (based on a careful ambient audio listening), a leg movement or a respiratory event (flow limitation, hypopnea, apnea) was discarded from the analysis.

Statistical analysis

The raw measures were transformed as relative changes (in %) from the individual baseline measurement performed 10 s before awakening, in order to (i) allow for comparisons between subjects who had different heart and respiratory measures in N3 stage, as well as within subjects when measures were taken at different N3 arousals, and (ii) to get rid of calibration problems. The clinical measures were compared between groups using chi-square tests for qualitative measures and Student's t tests for quantitative measures using SAS version 9 (SAS Institute, Cary, NC). Because the number of N3 arousals was different among participants, the measurements were adjusted for each subject and analyzed (patients vs. controls, pre- vs. post-arousal periods [within subjects], parasomniac vs. quiet arousals

[within-subjects]) using a mixed linear model on SAS V9, including interactions. When this first analysis yielded significant differences, adjusted Tukey-Kramer tests were performed for between-groups analysis. In order to estimate the pre-arousal, post-arousal, and the time of onset of the increase of heart rate and pulse wave amplitude curves after the stability period, we fitted the following non-linear model: $y(t)=a0+amplitude*(1-E^{-lamba\ (t-t0)})$ if $t>t0$; $y(t) = a0$ if $t<t0$; where y(t) is the value of the heart rate or the pulse wave amplitude, a0 is the mean pre-arousal value, amplitude is the difference between the mean post- and pre-arousal values, lambda is a parameter reflecting the speed of the change, and t0 is the time of onset of the increase.

RESULTS

Sample and sleep characteristics

The 20 patients with disorders of arousal suffered from isolated SW (n = 8, 40% of the sample), isolated ST (n = 4, 20%) or a combination of SW and ST (n = 8, 40%). Seizure-like activity was not detected during polysomnographic studies, and none of the patients had any personal or family history of seizure. The mean age, sex, body mass index and score of the Epworth sleepiness scale were not different between SW/ST and control groups (Table 1). Patients with SW/ST slept longer, had longer wakefulness after sleep onset, and more arousals from N3 despite lower sleep fragmentation (reduced number of arousals plus awakenings per hour of sleep), whereas other sleep measures were not different from those in controls (Table 1).

Autonomic changes surrounding N3 arousals in patients

There were 1 to 18 arousals from N3 sleep per patient (50 quiet and 55 parasomniac arousals) and 1 to 4 arousals from N3 sleep per control (38 quiet and no parasomniac arousals). The raw heart rate profiles across time (Figure 2) were best estimated using an S curve with a low and stable rate from the -10th to the -6th heartbeats and a gradual increase from the -4th heartbeat before the onset of N3 arousal. The estimates of the curve were a0 = 62.1 ± 0.9 beats per min, time of onset = 10.8 ± 0.2 s, amplitude = 23.7 ± 1.4 beats per min, and lambda = 6.8 ± 1.9 s, which indicated that the inflexion of the curve occurred 4 seconds before the arousal. Heart rates were higher (+33% on average) after arousal than before arousals (Table 2). A mirroring pattern was observed for the pulse wave amplitude (Figure 2), starting with a stable amplitude, a high amplitude during the stable N3 stage, followed by a gradual decrease in amplitude from the -4th pulse wave before the arousal to the +5th pulse wave, and finally a relatively stable, low amplitude from the 5th to the 15th pulse waves. The amplitude was greater (+57%) before arousals than after arousals onset, indicating vasoconstriction after arousals. The respiratory rate (+24%) and amplitude (+100%) were greater after arousals than before arousals (Table 2, Figure 3).

Comparison with controls

The raw heart rate curve across time (Figure 2) was best estimated in controls using an S curve with the following estimates: a0 = 65.4 ± 1.6 (higher than in the patient group, p = 0.02), time of onset = 10.3 ± 0.5 seconds (not different from patient group), amplitude = 21.4 ± 2.6 beats per min (not different from patient group), and

lambda = 6.4 ± 4.6 (not different from patient group). In controls, the heart rate and respiratory amplitude were greater after arousals than before arousals, the pulse wave amplitude was lower after arousals than before arousals, and the respiratory rate remained unchanged before and after arousals (Table 2). Patients had more marked changes than controls before vs. after N3 arousals for all measures (heart rate, pulse wave amplitude, respiratory rate and amplitude), as they had lower values before arousals (heart rate, respiratory rate) or higher values after arousals (heart rate, respiratory amplitude). The pulse wave amplitude was lower before arousals than after arousals.

Parasomniac vs. quiet arousals in sleepwalkers

The heart rate exhibited a greater increase from baseline to parasomniac arousals than from baseline to quiet arousals, because it was lower before parasomniac than before quiet arousals (Table 2, Supplemental Figure A). Conversely, the pulse wave amplitude decreased more after parasomniac arousals than after quiet arousals, because it was higher before (and was not different after arousals) parasomniac arousals than before quiet arousals. The respiratory rate increased after arousals in both groups, with a higher respiratory rate after parasomniac arousals than after quiet arousals (Supplemental Figure B). In contrast, the respiratory amplitude was not changed by the nature of the arousals.

Quiet arousals in sleepwalkers and in healthy controls

The autonomic profile associated with the 50 quiet arousals in sleepwalkers was compared to the profile in 38 quiet arousals in healthy controls. The heart rate was similar before and after the arousals in both groups, with a non-different increase

after the arousal (Table 2 and Supplemental Figure C). In contrast, the pulse wave amplitude (which was similar before the quiet arousals in the sleepwalking and in control groups) was lower after quiet arousals in sleepwalkers, hence the post-arousal drop was greater in the sleepwalking group. The respiratory rate (but not the respiratory amplitude) had a greater increase after the quiet arousals in sleepwalkers than in controls (Supplemental Figure D).

DISCUSSION

Main findings

In patients with SW/ST, the N3 arousals were associated with a 33% increase in heart rate, a 57% decrease in pulse wave amplitude (indicating a major vasoconstriction), a 24% increase in respiratory rate and a doubling of respiratory amplitude. Notably, tachycardia and vasoconstriction started 4 seconds before motor arousals. The same profile (tachycardia and vasoconstriction gradually increasing from the 4 s preceding arousal, post-arousal increase of respiratory amplitude, but no polypnea) was also observed, to a lesser degree, during the less frequent N3 arousals in control subjects. Parasomniac arousals were associated with greater tachycardia, vasoconstriction and polypnea than quiet arousals, with the same pre-arousal gradual increases in heart rate and vasoconstriction.

Autonomic reactions are associated with various types of arousals

Cortical arousals caused by acoustic stimuli (Catcheside *et al.*, 2002), periodic leg movements (Pennestri *et al.*, 2013), or apnea/hypopnea (Pitson and Stradling, 1998) are all associated with enhanced sympathetic drive (increased heart rate and blood

pressure as well as decreased pulse transit time). The highest response to noises has been observed in N3 arousals, as compared to waking (Griefahn *et al.*, 2008). Increases in cardiovascular markers are also observed during spontaneous arousals in young subjects (Bonnet et al., 2007), as found here in young subjects, with and without parasomnia. Arousals caused by noise, flow limitations, limb movements or nurse interventions were carefully discarded from the analysis, such that the remaining N3 arousals were apparently spontaneous.

Autonomic reactions during parasomniac arousals

Enhanced sympathetic drive is a normal reaction to any N3 arousal (Janackova and Sforza, 2008), but here this reaction was more increased in patients with SW/ST than in healthy controls, and was further increased when the arousal was associated with abnormal behaviours, facial expressions of surprise or fear, or simply sudden eye opening. Fisher et al observed that the heart rate increased after, but not before, the arousals onset (Fisher *et al.*, 1973), but the measures were averaged over 2.5 min before the N3 arousal, without paying attention towards the few seconds preceding the N3 arousal. Signs of intense autonomic discharge, such as mydriasis, tachycardia, tachypnea, flushing of the skin, and sweating, are part of the sleep terror phenotype and appear to be congruent with the apparent fear of the subject (American Academy of Sleep Medicine, 2014). Two groups found that heart rate increased after N3 arousals in patients with sleepwalking (Schenck *et al.*, 1998; Busek *et al.*, 2005) and in controls (Schenck *et al.*, 1998). Our results confirm these previous observations and extend the findings to vasoconstriction as well as respiratory rate and amplitude (not measured before) and to quiet N3 arousals in

patients with ST/SW (which contained a more marked vasoconstriction and polypnea than the quiet arousals in controls), suggesting that the sympathetic discharge associated with N3 arousals is higher in patients than in controls. It is here not possible to determine whether this increased cardiorespiratory activity is the consequence of an increased sympathetic activity or of a decreased parasympathetic activity, although the former seems more plausible than the latter.

Because patients have more frequent N3 arousals than controls, they have more frequent sympathetic activation. Such activation could have long-term deleterious consequences on the cardiovascular system. In this direction, Busek et al found an increased sympathetic response to standing up in sleepwalkers compared to controls, although the heart rate variability did not differ between groups across sleep and quiet waking (Busek *et al.*, 2005).

Autonomic arousals preceding motor arousals

Here, the sympathetic discharge started approximately 4 seconds before the motor arousal. When studying N3 arousals, Schenck et al. noted that the RR intervals on EKG were unchanged during N3 sleep, and then progressively decreased from the -3rd heartbeat prior to N3 arousal to the +2nd heartbeat after arousal (Schenck *et al.*, 1998). Busek et al. found an increased heart rate on average one second prior to arousals in sleepwalkers, possibly because they chose as onsets other markers of activation (increased respiration, EEG changes, eye movements) than just an EMG increase (Busek *et al.*, 2005).

What could evoke these autonomic arousals?

This autonomic arousal preceding spontaneous motor and cortical N3 arousal may reflect an internal stimulus (an alarming event) provoking the arousal. The nature of the alarming event is unknown, but one may imagine that it could be some troubling sleep mentation. Indeed, when not amnestic, most patients with SW/ST report a brief nightmarish (baby in danger, ceiling collapse, being buried alive, not finding the exit, intruders, tsunami, poisoned air) or concerning (unfinished homework, clothes to be washed) scene congruent with the parasomnia episode (Fisher *et al.*, 1974; Schenck *et al.*, 1989; Oudiette *et al.*, 2009; Mwenge *et al.*, 2013; Arnulf *et al.*, 2014). These threatening dreams in SW/ST contrast with the low frequency (34%) of emotions found in dream reports collected after provoked arousals from N3 in normal subjects (Cavallero *et al.*, 1992). One may wonder whether the dream scenario that occurs during N3 sleep (and that is poorly recalled upon awakening) does not gradually develop to the point of being so emotionally charged that it evokes an arousal. Conversely, one may also imagine the reverse scenario: a spontaneous activation of the autonomic nervous system occurring in N3 could then trigger the "troubling dream mentation", as some physical sensation can be incorporated into the ongoing dream scenario (Dement and Wolpert, 1958). In this direction, the SW/ST patients report frightening/threatening dream fragments (i.e., short time course) and not well-developed dream plots (as more commonly found in REM sleep). If true, patients with SW/ST should benefit from drugs that reducing sympathetic activity during sleep (e.g., alpha-blockers like prazosin), which are commonly used to alleviate the nightmares associated with post-traumatic stress disorder. A similar alert would be at play in patients with SW/ST and in healthy subjects (as both present sympathetic activation prior to N3 arousal), but the inability to fully, consciously awake patients with SW/ST

would prevent them from realizing that they just had a bad dream, and hence their inappropriate behaviour. Alternatively, this early autonomic arousal could be an external marker of the local, subcortical arousal preceding by 3-5 s the surface EEG arousal (Terzaghi *et al.*, 2009; Januszko *et al.*, 2016; Desjardins *et al.*, 2017). Both hypotheses are not mutually exclusive. It would be interesting to correlate the autonomic analysis with all these recent connectivity and stereo EEG analysis measures in confusional and non confusional arousals.

Parasomniac events triggered by external stimuli

It would be interesting to elucidate whether the onset of tachycardia and vasoconstriction shifts toward motor arousal onset (rather than prior to it) when N3 arousals are evoked by noise in patients with SW/ST and in controls. Indeed, sudden noise (or contact) may also trigger parasomniac episodes in predisposed subjects (Pilon *et al.*, 2008). Two types of abnormal N3 arousals can be considered: those precipitated by nightmarish or alarming sleep mentation (internal, mental trigger, with autonomic reaction preceding the motor arousal), and those precipitated by ambient noise, apnea, movement, contact, pain or esophageal reflux (external triggers, with autonomic reaction associated with the trigger). Both would result in inappropriate behavioural responses in patients and in appropriate behavioural responses (quiet arousal, repositioning, resuming sleep) in controls.

This study has several limitations, including the small number of patients and controls that were studied. This size limitation is counterbalanced by the manual measurements of more than 10,000 autonomic events and the careful exclusion of any artifact caused by movement, or arousal caused by noise, nurse intervention or

flow limitation. The measure of vasoconstriction via the pulse oximetry was here a surrogate, as the gold standard continuous measure of blood pressure is a finger plethysmography (e.g., Portapres®), a non-routine, expensive device, which may alter sleep continuity. Similarly, the measure of respiratory reaction was based on plethysmography band, which is not a calibrated measure of ventilation as obtained using a pneumotachograph. However, it would be difficult to obtain normal sleep in young SW/ST patients unused to wear a facial mask.

In conclusion, a sympathetic arousal comes 4 seconds before any motor or cortical arousal from N3 sleep, suggesting that an alarming event (possibly troubling sleep mentation or local, deep arousal) during sleep causes parasomniac (in SW/ST patients) or quiet N3 arousals (in healthy subjects and in SW/ST patients).

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Author contribution

NL, EA, PCS and MCS performed the measurements, JLG performed the statistical plan and analysis, SR supervised the autonomic measures, and IA organized the project, recruited the patients, supervised the analysis and obtained the funding. All coauthors contributed to the final analysis of data and to the article.

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Table 1 - Demographic and clinical characteristics of the patients with sleep terrors and sleepwalking episodes vs. healthy controls

Group	Patients	Healthy controls	P
No	20	20	
Age, y	26.8 ± 6.6	28.2 ± 8.8	0.57
Female sex, n (%)	10 (50)	10 (50)	
Body mass index, kg/m²	22.3 ± 3.6	23.5 5.4	0.44
Epworth sleepiness score, 0-24	9.9 ± 5.4	10.4 ± 7.2	0.81
Sleep measures			
Sleep onset latency	26.1 ± 18.0	25.6 ± 17.4	0.93
REM sleep onset latency	118.4 ± 58.4	136.6 ± 73.5	0.41
Sleep efficacy, %	88.3 ± 6.0	91.3 ± 5.7	0.11
Wake after sleep onset, min	67.6 ± 38.7*	42.8 ± 32.5	0.04
Total sleep time, min	501.7 ± 72.7*	435.6 ± 59.7	0.004
Sleep stages (% of total sleep time)			
N1 sleep	3.6 ± 2.4	3.45 ± 2.4	0.83
N2 sleep	50.3 ± 9	50.0 ± 5.9	0.91
N3 sleep	24.8 ± 7.9	25.2 ± 6.9	0.87
REM sleep	20.1 ± 3.9	18.9 ± 6	0.44
Arousal index	8.2 ± 4.7*	14 ± 6.0	0.002
Periodic leg movements index	0.5 ± 0.8	2.6 ± 4.9	0.07
Apnea/hypopnea index	2.6 ± 6.0	2.5 ± 3	0.91
Arousals from N3 sleep, n/night	5.6 ± 3.5*	1.8 ± 0.8	< 0.001

* $P < 0.05$ for a difference between patients and healthy controls

Table 2 - Autonomic measures before and after N3 (quiet or parasomniac) arousals in patients with sleepwalking episodes and sleep terrors, and in healthy controls

	Controls	Patients	Parasomniac arousals in patients	Quiet arousals in patients
N3 arousals, No	38	105	55	50
Heart rate, beats per min				
Before arousals	64.9 ± 0.8	60.7 ± 0.5[a]	57.2 ± 0.6[d]	64.7 ± 0.7
After arousals	79.3 ± 0.7[b]	80.9 ± 0.4[b]	81.4 ± 0.5[b]	80.5 ± 0.6
Before/after change	14.3 ± 1.1	20.3 ± 0.6[c]	24.2 ± 0.8[e]	15.8 ± 0.9
Pulse wave amplitude, microvolts				
Before arousals	150.7 ± 3.7	140.5 ± 1.6[a]	147.9 ± 2.2[d]	131.8 ± 2.4
After arousals	94.5 ± 3.3[b,d]	60 ± 1.4[a,b]	60.4 ± 1.9[b]	59.6 ± 2[b]
Before/after change	-56.2 ± 4.2[d,f]	-80.5 ± 2.3[c]	-87.5 ± 2.9[e]	-72.2 ± 3.1
Respiratory rate, cycles/min				
Before arousals	17 ± 0.6[d]	14.7 ± 0.3[a]	14.4 ± 0.4	15.1 ± 0.5
After arousals	17.7 ± 0.5	18.3 ± 0.3[b]	19.0 ± 0.3[b]	17.6 ± 0.4
Before/after change	0.6 ± 0.7[d]	3.6 ± 0.4[c]	4.5 ± 0.6[c]	2.5 ± 0.6
Respiratory amplitude, ~~mL~~				
Before arousals	0.79 ± 0.07	0.73 ± 0.04	0.75 ± 0.06	0.71 ± 0.07
After arousals	1.35 ± 0.06[b]	1.57 ± 0.04[a, b]	1.63 ± 0.05[b]	1.50 ± 0.05[b]
Before/after change	0.56 ± 0.1	0.83 ± 0.06[c]	0.88 ± 0.08	0.79 ± 0.08

The measures are the least squares means estimate ± standard error; [a]$p<0.05$ for a difference with controls, [b]$p<0.0001$ for a difference with the pre-arousal period, and [c]$p<0.05$ for an interaction between the groups and periods; [d]$p<0.05$ for a difference with quiet arousals, [e]$p<0.05$ for an interaction between the types of arousal (quiet or

parasomniac) and periods, Tukey-Kramer tests, and [f]$p<0.05$ for an interaction between quiet arousals (in controls vs parasomniac) and periods, Tukey-Kramer tests.

Figure legends

Figure 1 –A motor arousal from N3 is shown in a compact window (A) with polysomnography, including from top to down, 4 left (blue) and right (red) EEG channels, eye movements (LOC/A2; ROC/A2, black), chin muscle tone (EMG, green) over a 30 s epoch, (B) nasal (blue) pressure and oro-nasal (brown) thermistance, thoracic (velvet) and abdominal (green) plethysmography, heart rate (red) and pulse wave oximetry (green) over a 5 min period. The exact heart rate (C) and pulse wave amplitude (D) measures are then displayed in a wider window. The time of arousal (vertical dotted line) corresponds to the sudden onset of motor activity on the chin muscle.

Figure 2 - Changes in heart rate (upper panel) and pulse wave amplitude (a surrogate indicator of vasodilatation/constriction, lower panel) before and after N3 arousal (vertical black arrow) in patients with sleepwalking episodes or sleep terrors (solid blue line and circles) and in healthy controls (green dotted line with triangles). All post-arousal measures were different between groups.

Figure 3 –Changes from baseline, before and after N3 arousal, in respiratory rate (upper panel), respiratory amplitude (lower panel) in patients with sleepwalking/sleep terror (solid blue columns) and in healthy controls (dashed green columns).

Supplemental Figure A - Changes in heart rate (upper panel) and pulse wave amplitude (a surrogate indicator of vasodilatation/constriction, lower panel) before and after N3 arousal (vertical black arrow) in patients with sleepwalking or sleep

terrors episodes during parasomniac (solid blue line and circles) vs. quiet (orange dotted line with circles) awakenings from N3.

Supplemental Figure B –Changes from baseline, before and after N3 arousal, in respiratory rate (upper panel), respiratory amplitude (lower panel) in patients with sleepwalking/sleep terror during parasomniac (solid blue columns) vs. quiet (dashed orange columns) awakenings from N3.

Supplemental Figure C - Changes in heart rate (upper panel) and pulse wave amplitude (a surrogate indicator of vasodilatation/constriction, lower panel) before and after N3 quiet, non parasomniac arousals (vertical black arrow) in patients with sleepwalking or sleep terrors episodes (solid orange line and circles) vs. N3 arousals (green dotted line with triangles) in controls. There were no between groups differences for heart rate, but the post-arousal vasoconstriction was more marked in patients than in controls, despite the behaviour was quiet in the two groups.

Supplemental Figure D –Changes from baseline, before and after N3 arousal, in respiratory rate (upper panel), and respiratory amplitude (lower panel) in patients with sleepwalking/sleep terror during quiet arousals in sleepwalkers (solid orange columns) and in controls (dashed green columns). Note the higher respiratory rate (unlike respiratory amplitude) after the quiet arousals in sleepwalkers.

Fiure 1

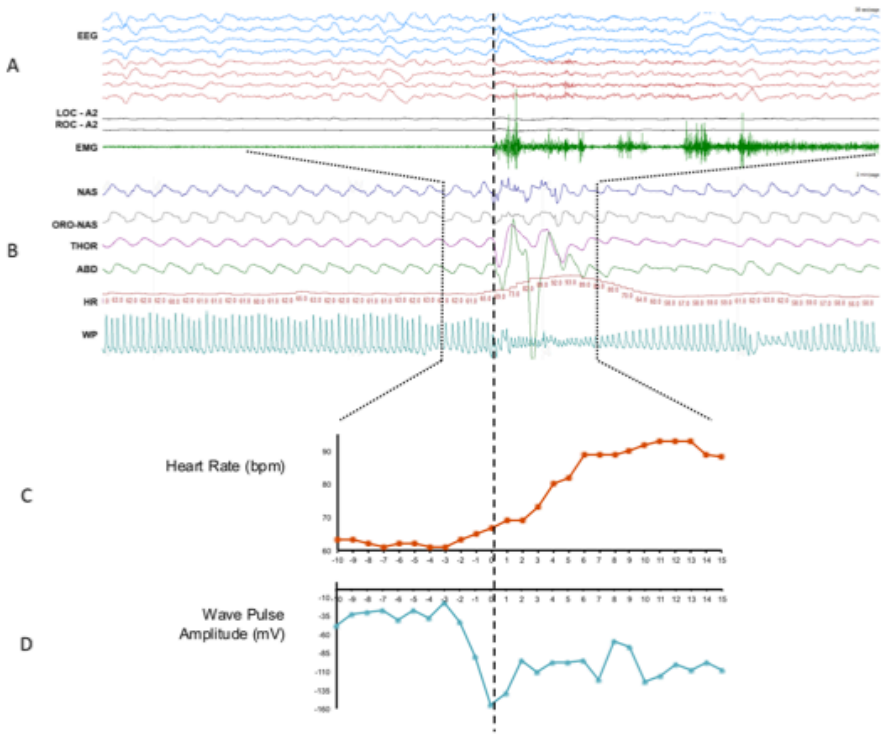

A
EEG
LOC - A2
ROC - A2
EMG
B
NAS
ORO-NAS
THOR
ABD
HR
WP
C
Heart Rate (bpm)
D
Wave Pulse
Amplitude (mV)

Figure 2

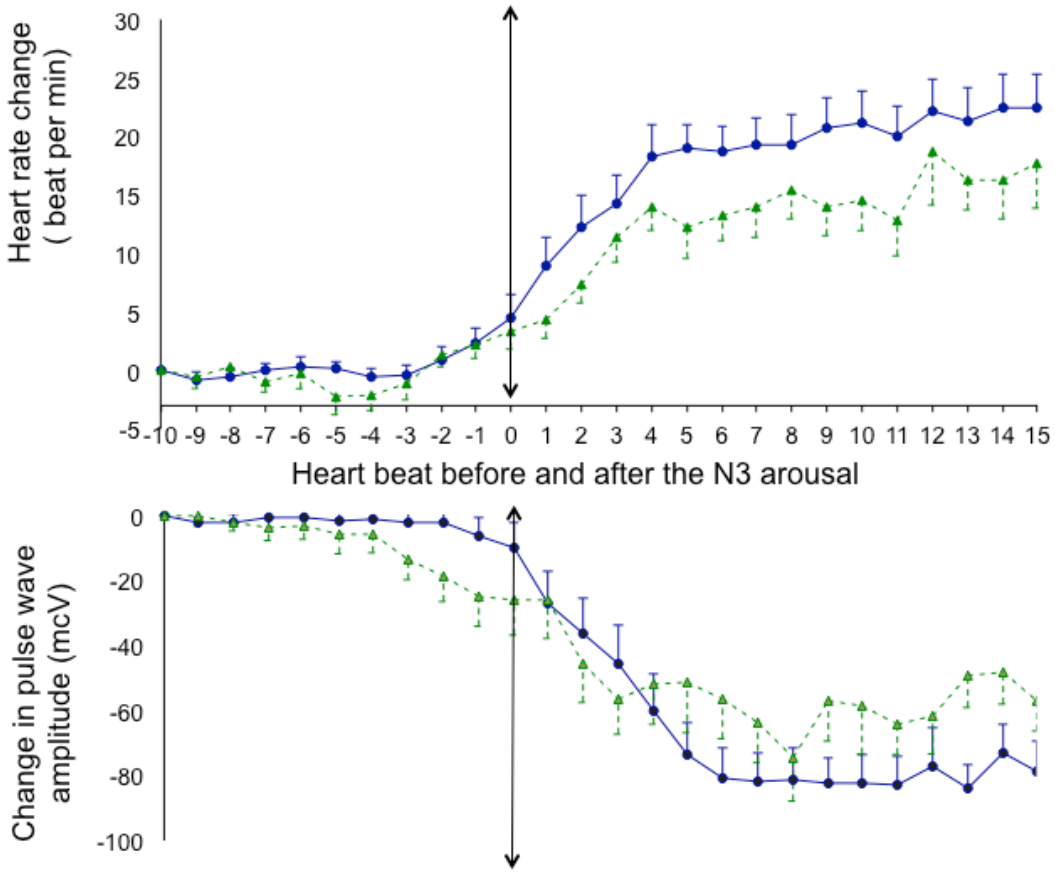

Heart rate change
(beat per min)
30
25
20
15
10
5
0
-5
-10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Heart beat before and after the N3 arousal
Change in pulse wave
amplitude (mcV)
0
-20
-40
-60
-80
-100

Figure 3

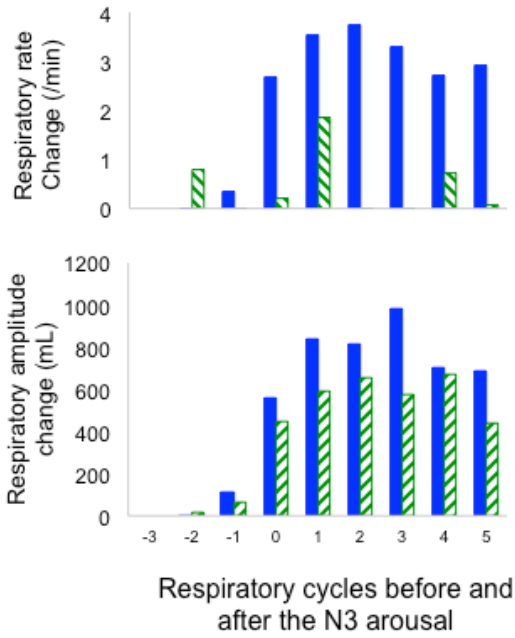

Respiratory rate
Change (/min)
4
3
2
1
0
Respiratory amplitude
change (mL)
1200
1000
800
600
400
200
0
-3
-2
-1
0
1
2
3
4
5
Respiratory cycles before and
after the N3 arousal

Figure A

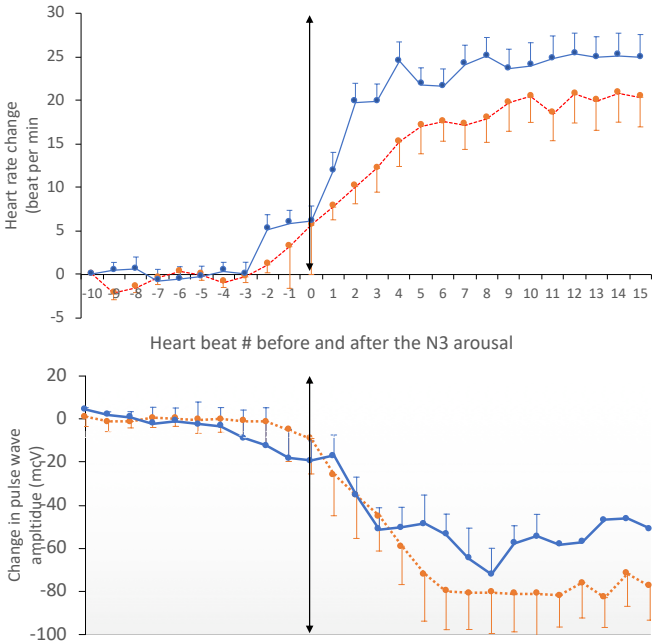

Heart rate change (beat per min
30
25
20
15
10
5
0
-5
-10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Heart beat # before and after the N3 arousal
Change in pulse wave amplitude (mcV)
20
0
-20
-40
-60
-80
-100

Figure B

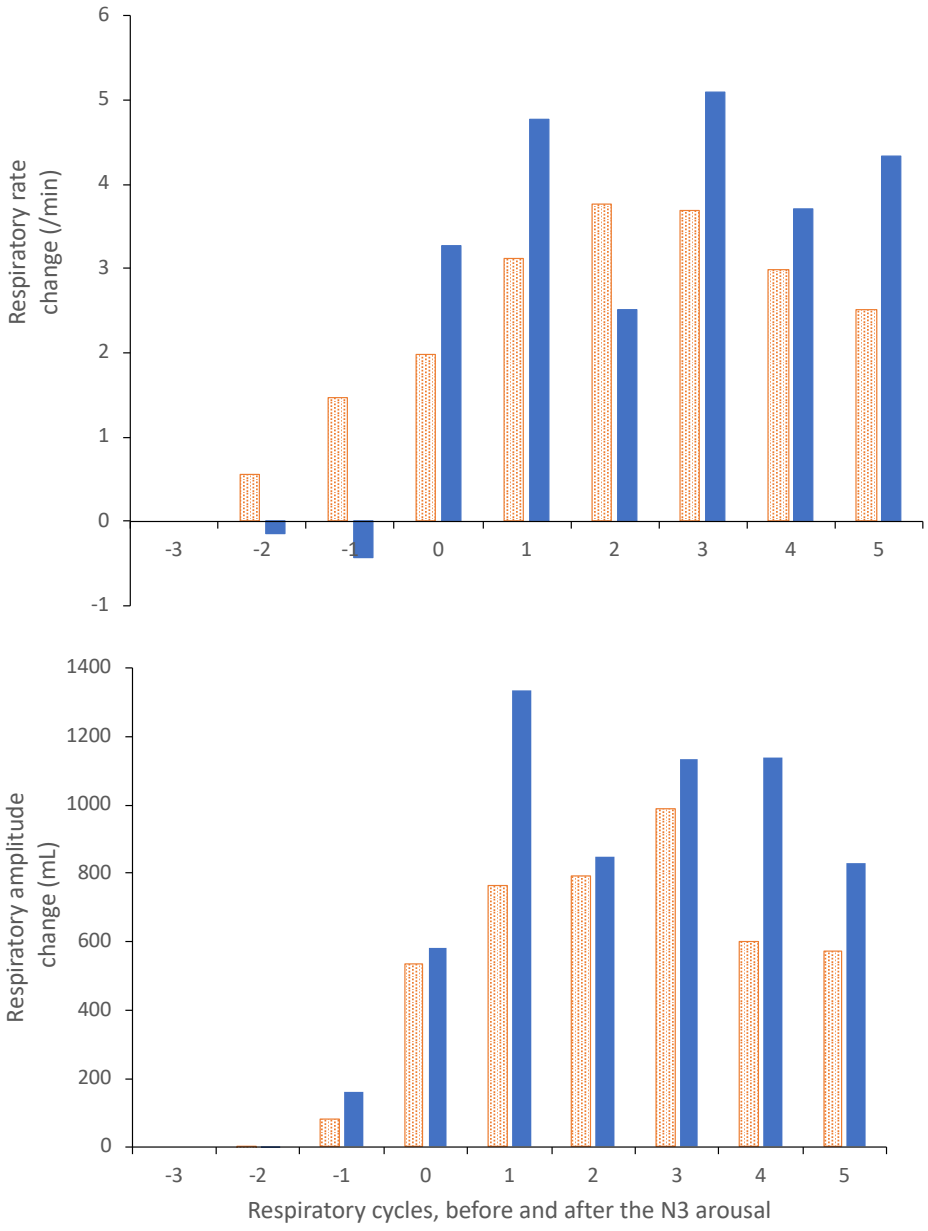

Respiratory rate change (/min)
6
5
4
3
2
1
0
-1
-3
-2
-1
0
1
2
3
4
5
Respiratory amplitude change (mL)
1400
1200
1000
800
600
400
200
0
-3
-2
-1
0
1
2
3
4
5
Respiratory cycles, before and after the N3 arousal

Figure C

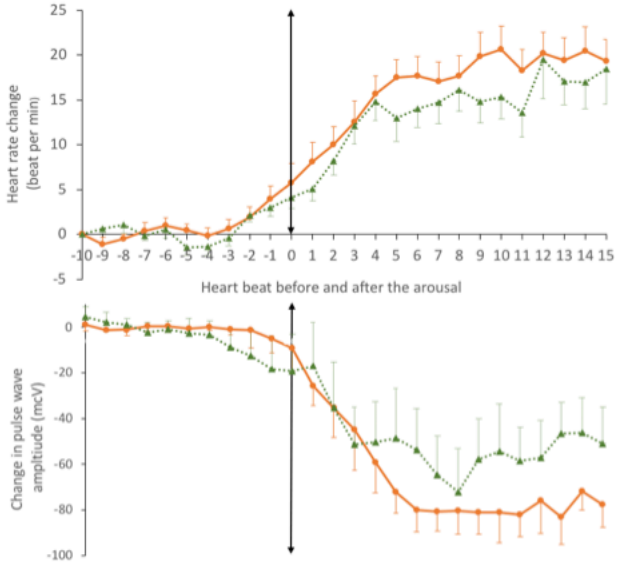
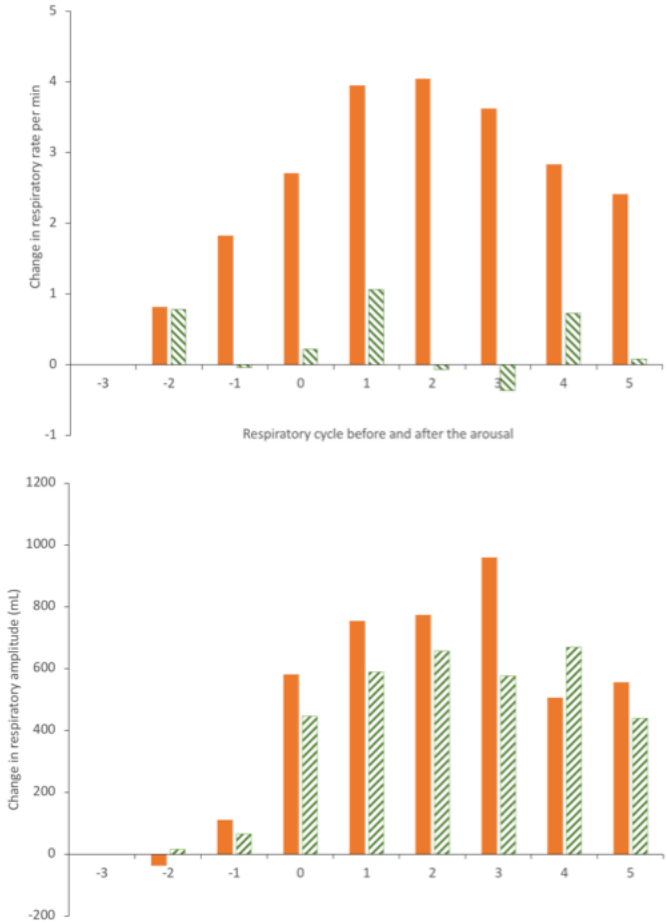

Heart rate change (beat per min)
25
20
15
10
5
0
-5
-10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Heart beat before and after the arousal
Change in pulse wave amplitude (mcV)
0
-20
-40
-60
-80
-100

Figure D


Change in respiratory rate per min
5
4
3
2
1
0
-1
-3
-2
-1
0
1
2
3
4
5
Respiratory cycle before and after the arousal
Change in respiratory amplitude (mL)
1200
1000
800
600
400
200
0
-200
-3
-2
-1
0
1
2
3
4
5