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SUMMARY

Human foetuses and new-borns smile first during sleep, before they smile while awake and interacting with caregivers. Whether smiling persist during adult sleep, and express inner joy, is yet unknown. Smiles were looked for during night-time video-polysomnography combined with electromyography of the *zygomatic* and *orbicularis oculi* muscles in 100 controls, 22 patients with sleepwalking, and 52 patients with rapid eye movement (REM) sleep behaviour disorder. Autonomous reactions (heart rate, level of vasoconstriction) and the presence of rapid eye movements were examined during smiles/laughs. On visual examination of the face video clips synchronous with *zygomatic* contraction, 8% of controls smiled asleep (7% in REM sleep and 1% in non-REM sleep). Some patients with sleepwalking also smiled and laughed during N2 sleep and N3 parasomnia. Half of patients with REM sleep behaviour disorder smiled, and one-third laughed, mostly during REM sleep. The 173 happy faces included mild smiles (24.8%), open-mouth smiles (29.5%) and laughs (45.7%). More than half of smiles were the Duchenne (genuine) type, including an active closure of the eyelids. Approximately half of smiles and laughs were temporally associated with rapid eye movements. There was no increased heart rate variability during smiles and laughs. Two scenic behaviours including smiles and laughs suggested that the happy facial expression was associated with a happy dreaming scenario. Smiling and laughing occasionally persist during adult sleep. There are several lines of evidence suggesting that these happy emotional expressions reflect a true inner mirth.

Abbreviations: EMG = electromyogram; NREM sleep = non-rapid eye movement sleep; REM sleep = rapid eye movement sleep; REMs = rapid eye movements; RBD = rapid eye movement sleep behaviour disorder.

INTRODUCTION

The facial expressions of happiness (smiling, laughing), sadness, surprise, fear, anger, and disgust are recognized as a universal non-verbal language found in all human ethnicities but also in some non-human primates (Ekman and Friesen, 1969). Darwin stipulated that some facial expressions were not learned but biologically determined, including specific inborn emotions that are expressed even in the neonate and may serve a basic survival function (Darwin, 1897/1965). Among these expressions, smiling is an early social and emotional behaviour. An "endogenous" smile appears *in utero*, as early as during the 15th week of gestation, with more consistent smiles between 18 and 20 weeks (Piontelli, 2010). Sleep monitoring in premature infants and neonates indicates that smiling occurs during active sleep, the precursor of rapid eye movement (REM) sleep (Emde et al., 1971, Messinger et al., 2002). After birth, smiles often occur during sleeping states without obvious external stimulation. The infant smile is a powerful tool to attract the attention of the caregivers, driving them to become involved in an interaction. Communicative smiles in the presence of the caregiver are observed. In parallel, the frequency of sleep-associated smiles decreases between the 3rd and 6th months, but a few sleeping smiles are observed after 1 year (Kawakami et al., 2009a).

In adults, *zygomatic* activity is highly correlated with positive affect in awake subjects (Brown and Schwartz, 1980). However, the activity of facial muscles during sleep has rarely been studied through electromyography (Bliwise et al., 1974). In six healthy subjects, the phasic activity of facial muscles, including the *corrugator* (involved in negative emotional expression*)*, *zygomatic* (involved in positive emotional expression), *masseter, orbicularis oculi*, *depressor anguli oris* and *frontalis* muscles, persisted while asleep, with a higher frequency during REM than NREM sleep (Rivera-Garcia et al., 2011). In addition, some facial muscles tended to co-contract during REM sleep, producing a pattern consistent with emotional expression during waking (Rivera-Garcia et al., 2011). However, these studies did not include concomitant video monitoring. Consequently, they could not evaluate the presence of visually observable emotional expressions during sleep.

We aimed to examine whether visible smiling and laughing would persist during normal adult sleep, using a large group of subjects and combined video, sleep and EMG monitoring. In addition, we aimed to measure whether smiling and laughing would have the characteristics of emotional expressions, as indicated by the concomitant autonomic reactions, association with rapid eye movements and presence of Duchenne (genuine) smiles (Duchenne de Boulogne, 1862-1990). Furthermore, we studied smiles and laughs in models of enacted dreaming, including REM sleep behaviour disorder (RBD) and NREM parasomnias (sleepwalking). We hypothesized that facial expressions would be more frequent in these parasomnias due to imperfect normal muscle atonia in RBD and to complex behaviours in sleepwalkers and that some of the concomitant behaviours would help to determine whether there is a concomitant pleasant dream scenario.

METHODS

Participants

The 174 consecutive participants (men, N = 94; women, N = 80) were recruited in the Sleep Disorders Unit of the Pitié-Salpêtrière university hospital between February and June 2016. All adults referred to the unit for a sleep study and consenting to take part in the study (i.e., to have facial EMG in addition to the routine sleep and video recording) were included, except those with untreated sleep apnoea and with sleep-related motor disorders. Please note that video monitoring during the night is a routine, consistent procedure in our sleep unit, meaning that all subjects are videotaped, even those with no movement disorder or parasomnia. The sleep disorders were defined in accordance with the International Classification of Sleep Disorders-3 (American Academy of Sleep Medicine, 2014). The participants were split into two groups, with and without parasomnia. In the parasomnia group, the patients with RBD had to meet the international criteria, including i) repeated episodes of sleep-related vocalization and/or complex motor behaviours; ii) that these behaviours were documented by polysomnography to occur during REM sleep or, based on clinical history of dream enactment, were presumed to occur during REM sleep; iii) that polysomnographic recording demonstrated REM sleep without atonia (defined here as the presence

of enhanced tonic chin muscle tone during more than 18% of REM sleep epochs (Frauscher et al., 2012); and iv) that the disturbance was not better explained by another sleep disorder, mental disorder, medication, or substance use. Idiopathic RBD was defined by the absence of any neurological disorder at time of inclusion, after an interview, a neurological examination and cognitive tests. The patients with NREM parasomnia had to meet the international criteria of arousal disorders, including 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 v) that the disturbance was not better explained by another sleep disorder, mental disorder, medical condition, medication, or substance use. Patients with sleepwalking (same criteria plus ambulation and other complex behaviours out of bed), night terrors (with episodes typically beginning with an alarming vocalization, intense fear and signs of autonomic arousal) or both (as the two phenomena are frequently associated in adults) were grouped in a single group labelled “sleepwalking”. The non-parasomnia group included all subjects without REM and NREM parasomnia (none of the above criteria) and without sleep-related motor disorders. There were healthy subjects (no sleep complaint or sleep disorders, recruited and paid to take part in another study), patients with a complaint of insomnia, patients with adequately treated sleep apnoea referred for controlling the treatment efficacy, patients with Parkinson's disease and no RBD or sleep complaint who volunteered for another research trial, patients with narcolepsy (but without RBD), and patients with idiopathic hypersomnia. All participants provided written or oral informed consent (after the study was explained to them by the research assistant and physicians) for this study, depending on their groups. In patients referred for exploring their sleep disorders, this study consisted of adding surface EMG of two facial muscles to the routine recording and completing a test on facial expression, which was considered a non-invasive, mild supplementary exploration, for which the local ethics committee (Comité de Protection des Personnes Ile-de-France VI) waived written consent but asked for verbal non-opposition of patients, consigned in the subject file. Healthy volunteers, patients with PD (with and without RBD), with idiopathic RBD and with sleepwalking/night terrors provided written consent for various studies approved by the local ethics committee (Comité de Protection

des Personnes Ile-de-France VI), including the ICEBERG (NCT02305147), HYPNOSOM (NCT02648568) and AEP programs and consented to the additional study of their facial expression during sleep. Only healthy volunteers were paid for participation. All participants provided consent for their face to be studied on video by researchers, but not to be shown in a medical journal, except for a few participants, but they provided consent for a scientific illustrator (Juliette Rey) to draw their face (without changing its expression) for illustration purposes, with changes rendering it unrecognizable.

Questionnaires

The participants were interviewed by a sleep neurologist who performed the diagnosis of their disorder (if any) following the international criteria after interview, clinical examination and review of the sleep tests (American Academy of Sleep Medicine, 2014). Participants completed the Epworth sleepiness scale (Johns, 1991) in the evening and a dream report the next morning (a free, written report of any dream mentation that they would recall upon final awakening), plus a general question about the pleasantness of their dream recall ("was your dreaming pleasant, unpleasant, neutral?"). Only the answers about the pleasant/unpleasant aspect of the recalled dreams were analysed here. Participants then underwent the condensed Ekman test for emotion recognition (Ekman and Friesen, 1976); they were required to choose one among seven expressions (anger, happiness, fear, sadness, disgust, surprise or neutral emotion, 5 pictures per expression) when looking at 35 black-and-white face pictures issued from 5 different characters. The score corresponded to the number of correctly identified emotions (0–35). This test allowed to determine whether no participant was deficient in recognising emotions, and to roughly check whether they displayed facial emotions (and especially smiling) in mirror during wakefulness.

Sleep evaluation

All participants underwent one or two successive video-polysomnographies, including Fp1-A2, C3-A2 and O1-A2 electro-encephalographic derivations (EEG),

bilateral electrooculography (EOG), nasal pressure and respiratory effort monitoring, tracheal sound recording, electrocardiography, pulse oximetry, EMG recording of the *levator menti* and bilateral *tibialis anterior* muscles (Compumedics Ltd, Australia). In addition, 4 surface electrodes were placed on *orbicularis oculi* and *zygomatic* muscles with a bipolar montage (Figure 1). The facial muscle calibration was performed in awake subjects during the Ekman test and a free conversation with the research assistant in order to evoke some expressions of happiness (smiles and laughs). The night behaviours and facial expression were filmed via infrared video cameras (one camera allowed a general view of the sleeper's full body, and a second was focused on the face), and a microphone captured the ambient sounds. The sleep stages, arousals, and respiratory and motor events were scored based on visual inspections of 30-s epochs according to standard criteria.(Iber et al., 2007) A REM sleep epoch was considered REM sleep without atonia if more than 50% of the epoch contained an enhanced EMG activity of the *levator menti* that was at least two times greater than the corresponding lowest activity during NREM sleep (Iber et al., 2007).

Evaluation of facial expression during sleep

The video observations were driven by the visual inspection of the *zygomatic* muscle activity on the EMG, which was measured in all subjects during the full night. An activation of the *zygomatic* muscle was defined as any waveform exceeding the baseline activity by 500% and lasting longer than 0.1 sec (Rivera-Garcia et al., 2011), a sub-clinical threshold that was found sufficient to catch any movement of the lips. The concomitant video was visually inspected during any muscle activation, which allowed to recognise unilateral lip raising, bilateral lip raising, and bilateral lip raising with an active closure of the eyelids. EMG activations without any observable event were discarded. Because smiles are defined by bilateral lip corner raising, a unilateral zygomatic EMG activation was sufficient to look at the concomitant face on the video and observe whether this EMG activity resulted in a smile or not. The video could not be fully examined in some patients sleeping face down or under the sheets. One may note that applying 6 additional EMG electrodes to the face (in addition to the electrodes of

the *mentalis* muscle, of electrooculography, of the Fp1 and ground EEG electrodes on the forehead, the pressure sensor plus oral thermistor within the nostrils and in front of the mouth) was sometimes perceived as uncomfortable by the sleeper and could blur the visualization of the facial expression. Consequently, we first monitored the left and right *zygomatic* muscles in the same patient and night (N = 12), then monitored the left (N = 52) or right (N = 78) *zygomatic* muscle in the next series of patients, and eventually monitored a combination of the *zygomatic* and *orbicularis oculi* muscles on opposite sides of the face (N = 88). The objective of monitoring the *orbicularis oculi* muscles was to catch concomitant proofs of Duchenne genuine smiles. The video and concomitant polysomnography signals were stored together. The video clips were scored using the following rating scale (Figure 1): i) compared to an absence of mouth movement, the unilateral raising of a lip corner (without concomitant raising of the contralateral lip corner) was scored as E1, a smile with raising of both lip corners as E2, a smile with raising both lip corners plus opening of the mouth as E3, and a full laugh as E4 (laugh markers included a shrug of the shoulder, a recognizable vocalization of breaking into a peal, and a belly contraction, visible on the abdominal belt). Simple twitches refer to E1 events. Most E1 events were short lasting (< 0.5 sec), whereas the majority of happy expressions lasted from 0.5 to 6 sec. The video clips were scored by two independent scorers. The video clips were discarded from the analysis when no consensus between the scorers could be reached. The Facial Action Coding System was not used here because it has not been developed for sleeping subjects with eyes closed (Ekman et al., 2002). The number of each of the 4 types of events was divided by the time asleep, as was the time spent in the sleep stage (N1, N2, N3 of NREM sleep, and REM sleep) in which it occurred, in order to develop an index of events per hour slept. We also measured the concordance between rapid eye movements (isolated and in bursts) and smiles. Because we needed to illustrate the scoring methods and findings but could not always display identifiable faces (to preserve the anonymity of the patients), a scientific illustrator (Juliette Rey, Estienne Art School, Paris) drew the facial expression asleep (without the electrodes) but rendered the faces unidentifiable without losing the expression. The two patients in the Video Clip#1 and #2 have expressly given their authorization for their video-clips to be published.

Correlates of happy emotional expressions during sleep

We indirectly investigated whether or not these visible smiles and laughs reflected an inner emotion, as it was not possible to awaken the subjects immediately after the smiles or laughs to get their mental recall, due to the rare and unpredictable occurrence of smiles and laughs. For this purpose, we looked for Duchenne smiles, which include smiling plus raising the upper part of the cheeks, produced by a contraction of the *orbicularis oculi* muscles. During wakefulness, Duchenne smiles are proposed to carry a true feeling of happiness (Johnson et al., 2010), in opposition to isolated raising of bilateral lip corners (which may correspond to 'polite' smiles). For this purpose, we measured the frequency of bilateral raising of the lip corners associated with concomitant EMG activation of the *zygomatic* and *orbicularis oculi* muscles or, in subjects without monitored EMG of the *orbicularis oculi,* with bagging or wrinkling the skin below the eye as well an active closure of the eyes (a dimple was visible in the lower eyelid and the basis of the eyelashes dug in, causing crows' feet). This aspect of the Duchenne smile was double-checked by two independent scorers, and only smiles scored as clear Duchenne smiles by both scorers were kept in the analysis.

In addition, we looked for autonomic activity concomitant with smiles and laughs while asleep in order to estimate whether those behaviours would be associated with changes in the orthosympathetic vs. parasympathetic tone. Indeed, some strong negative emotions are associated with increased heart frequency and skin temperature, although this is less consistent for happiness than for anger (Ekman et al., 1983). We measured the heart rate on the ECG and the pulse wave amplitude (vasoconstriction vs. vasodilatation, an indicator of the sympathetic vs. parasympathetic tone) before vs. during *zygomatic* muscle contraction. The measure started 5 seconds before the event, as this is a common time window for a baseline analysis of heart rate changes before and after motor events such as periodic leg movements (Pennestri et al., 2013). Because rapid eye movements (REMs) in REM sleep are temporally locked with the activity in the amygdala (Calvo and Fernández-Guardiola, 1984, Miyauchi et al., 2009, Andrillon et al., 2015, Corsi-Cabrera et al., 2016), we measured how frequently REMs

exactly overlapped the *zygomatic* muscle activity in REM sleep. Eventually, we looked for behaviours associated with smiles and laughs to find scenic behaviours directly indicative of pleasant or unpleasant mental scenarios.

Statistical analysis

The clinical and sleep measures were compared between groups using chi-square tests for qualitative measures and analysis of variance for quantitative measures adjusted for age (because it was different between groups), using the software R (R Core Team, 2016). If significant ($P < 0.05$), post hoc tests with the Bonferroni correction were performed between two groups using a P level below 0.0166 (0.05/3). As the analysis of face expression required 20 comparisons (within groups and between sleep stages, between simple twitches and happy expressions, and then between groups), we considered that $P<0.0025$ was significant (0.05/20, to avoid type A error). Statistics were performed with subjects as units of analysis (Table 1 and 2) and with nights as units of analysis (Table 3, which means that the subjects having slept two nights produced two values).

RESULTS

Characteristics of the sample

The non-parasomnia group (N = 100 subjects) included healthy subjects (N = 18) and patients with adequately treated sleep apnea referred for controlling the treatment efficacy (N = 29), with idiopathic hypersomnia (N = 18), with narcolepsy without RBD (N = 6), with insomnia (N = 3), with suspected parasomnia later ruled out (N = 11) and with Parkinson's disease without RBD (N = 15). No one had any sleep-related movement disorder, as checked by interview and video and sleep monitoring. Except the patients with Parkinson's disease, who took levodopa and dopamine agonists, no patients in the control group took any psychotropic drug. The sleepwalking group contained 22 adult patients, and the RBD group (N = 52) included patients with idiopathic RBD (N = 26) or narcolepsy (N = 7) and Parkinson's disease (N = 19). Patients slept one (n = 118) or two (n = 56) nights in

the sleep disorder unit, yielding 230 nights studied for facial expressions. Patients with RBD were older than non-parasomniac subjects, who were themselves older than sleepwalkers, but the sex ratio did not differ among the three groups (Table 1). The sleepiness scores were globally different between the three groups, with no further between-pairs significant differences. The ability to assess emotion on the Ekman test of 35 faces was similar across groups.

Smiles and laughs

In the whole sample, 173 happy expressions were found, including 43 (24.8%) mild smiles, 51 (29.5%) open-mouth smiles and 79 (45.7%) laughs. In 76.8% of the 173 smiles and laughs, concomitantly enhanced EMG activity in the chin muscle was noted. In the non-parasomnia group, smiles while asleep were rare and mostly restricted to REM sleep (8% of subjects, but one healthy volunteer smiled in N2 sleep, Figure 2) and laughs were absent (Table 2). In the sleepwalking group, rare smiles were observed during N2 sleep and REM sleep (Figure 2), and one patient laughed, both in N3 sleep (during a parasomnia event) and in REM sleep. The frequency of happy expressions per time asleep did not differ between NREM and REM sleep (Table 3; P = 0.35). Because this laugh during an N3 parasomniac event was unexpected, we later paid attention to this phenomenon among all adults with NREM parasomnia undergoing videopolysomnography during the next 18 months and found 5 patients among 120 (for a total of 6/142, i.e., 4%) with sleepwalking who smiled while “half-asleep” during the N3 parasomnia (Video clip 1). These new 5 patients were not included in the present analysed sample and were not equipped with zygomatic EMG. In the RBD group (Table 3), the full expressions of happiness, including mild smiles (Figure 2), open-mouth smiles and laughs (Video clip 2) were frequent, whereas unilateral lip raising was rarer (P = 0.0001 when compared with E2+E3+E4 events), even when hypothesizing that half of the unilateral lip raising was missed in patients with unilateral EMG sensor (P = 0.0009 when compared with E2+E3+E4 events). The frequency of all happy expressions per sleep time (unlike unilateral lip raising) was higher in REM than in NREM sleep. The index of unilateral lip raising was not different between groups, regardless of the sleep

stage. In contrast, patients with RBD had a greater index of happy expressions during REM sleep than sleepwalkers and non-parasomniac subjects had. They also had a greater index of happy faces during NREM sleep than non-parasomniac subjects but not compared to sleepwalkers. There were no differences in the joyful events index during REM sleep (P = 0.49) and NREM sleep (P = 0.17) between the 26 patients with idiopathic RBD and the 19 patients with Parkinson disease and RBD.

Evidence suggesting that happy faces reflect happiness

Half (52.5%) of smiles were “Duchenne” smiles (Table 1 and 2). During 79 laughs, 41 (51.9%) were associated with shrugging of shoulders and 45 (57%) with either the characteristic sound of a peal of laughter or, more rarely, with concomitant verbal (non-intelligible, but with a joyful affective prosody) utterances. Smiles and laughs were concomitant with REMs in REM sleep, with 70.5% occurring in the same 30-second epoch and with exactly simultaneous rapid eye movements in 57.7%. The heart rate frequency did not change from 5 seconds before the beginning of smiles and laughs to 5 seconds after, whether in patients with RBD (who may have an autonomic dysfunction) or in the other groups (who had no autonomic dysfunction). For the scenario associated with happy faces asleep, it was obvious (due to scenic concomitant behaviour) only in a patient smiling and laughing during an N3 arousal (Video-clip 1: a story of a cake placed in a basket, with potential candidates to eat it, at 2 AM). A patient with RBD first smiled and spoke, then laughed, and then sent a kiss, which suggested a pleasant associated dream. In the other cases, there was no speech or moaning, so it was not possible to infer the inner scenario. There was no smiling or laughing during visibly unpleasant scenic behaviours (including fighting, fracases, or fear shouting). Among 113 questionnaires collected on dream recall the following morning, 37 included dream stories, and 64 included recalls of emotion (including the recollection of having dreamt, having forgotten the content). The recalled emotions were pleasant (N =19), unpleasant (N = 18) and neutral (N = 27), according to the subjects, with no between-groups differences (Table 3). The presence of happy emotional faces during sleep was not associated with more pleasant dream recalls in the morning than their absence (P = 0.58).

DISCUSSION

General finding

This study indicates that smiles are not restricted to neonate sleep but persist during adult human sleep. Indeed, 8% of 100 subjects without parasomnia smiled asleep, 7% in REM sleep and 1% in N2 sleep. Rare patients with sleepwalking also smiled and laughed during N2, REM sleep and during N3 parasomnia. Half of patients with RBD smiled, mostly but not exclusively during REM sleep, and one-third laughed during REM sleep. The 173 happy facial expressions collected here included mild smiles (24.8%), open-mouth smiles (29.5%) and laughs (45.7%). More than half of smiles were Duchenne type, including an active closure of the eyelids. Approximately half of smiles and laughs were temporally associated with REMs. There was no increase in heart rate variability during smiles and laughs. Two scenic behaviours (during sleepwalking and during RBD) concomitant with smiles and laughs suggested that happy facial expressions were associated with pleasant dream scenarios.

Smiling and laughing in RBD

Smiles and laughs were expected in patients with RBD, as non-violent and pleasant behaviours exist during REM sleep in these patients, although they are less frequent than violent and aggressive behaviours (Oudiette et al., 2009a). Indeed, two patients with RBD laughed in the video clips of this series, showing scenic, pleasant behaviours during REM sleep: a man whistled, said, “Show a leg!”, laughed and concluded “Nobody, as usual!”; whereas a woman with RBD chatted with a neighbour and laughed. In addition, 4 spouses among 60 patients with Parkinson’s disease reported that their spouses occasionally laughed during RBD (Oudiette et al., 2009a). Among 67 patients with RBD and a video camera focused on the face, 21% laughed during REM sleep, despite most having concomitant depression and facial parkinsonian hypomimia during wakefulness (Siclari et al., 2011). In this direction, we previously observed in 47% of 55 patients with Parkinson’s disease that their awake parkinsonian hypomimia disappeared during RBD with a restoration of various facial expressions, in parallel with a

similar restoration of speed, strength and smoothness of limb movements (De Cock et al., 2007). Here, the frequency of joyful expressions was similar in patients with idiopathic RBD (who have not yet any hypomimia during wake), and in patients with Parkinson's disease plus RBD, who have a severe movement disorder and a reduced facial expression awake. This result supports previous works having suggested that the motor signature of RBD is the same across disorders (Oudiette et al., 2012), and that movements and face expressions are produced by different networks asleep than awake (Arnulf, 2012). Several lines of evidence (provided by movement analysis, functional imaging and deep brain recordings) suggest that during RBD, the activation of muscles is driven by the motor cortex and bypasses the basal ganglia (De Cock et al., 2007, Mayer et al., 2015, Hackius et al., 2016).

In 65 patients with RBD, apparently positive emotions were observed on the faces of 5% of the patients, compared with 30% displaying apparently negative emotions (Oudiette et al., 2012). The present study indicates that among patients with RBD, half of nights contain smiles and laughs, which is more frequent than reported during the previous observational studies of patients with RBD. The monitoring of *zygomatic* muscle activity combined with a video focused on the face may explain this difference, given that brief smiles can be missed during the video-clip observation. All in all, it suggests that happy expressions are displayed by a majority of patients with RBD.

Smiling and laughing during normal REM sleep

Subjects without RBD (including non-parasomniac subjects and sleepwalkers) also displayed happy emotional expressions during sleep in our study. However, these events were rare, since only 9 smiles and laughs were observed during 121 nights in non-parasomniac subjects and 4 during 38 nights in sleepwalkers. This low (0.012 events/h) frequency contrasts with the high frequency of smiles recorded in human neonates. The frequency of smiles is 1.6 smiles/h of apparent REM sleep (no concomitant sleep monitoring) in 5-8-month-old foetuses as observed using ultrasound echography (Kawakami et al., 2009b), 4-30/h of apparent REM sleep in preterm neonates (Emde and Harmon, 1972, Messinger et

al., 2002), and 4-19 smiles/h in full-term neonates (Messinger et al., 2002, Kawakami et al., 2009a), i.e., at least one hundred times more frequent than found here in normal adult REM sleep. However, patients with RBD reached a smile/laugh frequency (1.75/h of REM sleep) close to that of foetuses but lower than that of neonates, suggesting that the frequency difference between neonates and normal adults is likely to be caused (in part) by increased REM sleep atonia during post-term development. Their neural correlates might be however different.

Smiling and laughing during non-REM sleep

Notably, we observed 11 smiles in N2 sleep and 1 in N3 sleep, plus 5 episodes of smiles and laughs during N3 sleep. These happy faces were mostly observed in patients with parasomnia, including sleepwalking and RBD. We are not aware of any previous description of sleepwalkers smiling and laughing during NREM parasomniac events, as most patients with arousal disorders display behaviours suggestive of surprise, fear or confusion, often in link with brief unpleasant dreaming scenario (Oudiette et al., 2009b). However, it is possible that many sleepwalkers do smile or laugh during N3 arousal, but that such phenomena had been unnoticed because they did not disturb the partner's sleep. Surprisingly, the patients with RBD had a higher frequency of smiling during NREM sleep than did the non-parasomniac subjects and sleepwalkers, which suggests that their motor dyscontrol during sleep may extend to eliciting phasic motor activity in NREM sleep or that the central pattern generator producing happy emotional expressions is also enhanced in NREM sleep. Phasic motor activity may occasionally occur during NREM sleep in patients with RBD (Miguel and Arnulf, 2017), although this is as frequent as in healthy controls (Mayer et al., 2008). Eventually, one healthy control smiled during NREM N2 sleep. This result was unexpected but suggests that some phasic, coordinated motor activity occurs, although rarely, during NREM sleep.

Do sleep-associated smiles and laughs result from automatic muscle twitching?

The question of whether these numerous smiles and laughs performed asleep reflects an internal pleasant emotion or whether they are automatic, non-emotional expressions is crucial. Foetuses and neonates cannot verbally report any emotion after having smiled while asleep. Moreover, they may smile while asleep not because they are happy but as a way to train and develop this patterned behaviour underlying an important non-verbal language competence, useful for attaching to and interacting with adults when awake. Similarly, neonates, unlike adults, display abundant muscle twitches during REM sleep (including abundant twitching of the whiskers in rats), which are brainstem generated and do not involve any concomitant activation of the motor cortex (Blumberg et al., 2013). Twitching of facial muscles during adult REM sleep (independent of any emotional expression) is a common observation in adult sleep, especially on the *mentalis* muscle (Bliwise et al., 1974), which is routinely monitored in sleep units. Unilateral twitches of the *zygomatic* muscle during adult REM sleep have also been reported (Rivera-Garcia et al., 2011), although the absence of video monitoring did not allow determination of whether the twitches were associated with face expression. Here, we observed that these unilateral twitches led to brief, unilateral lip corner raising, without any concomitant expression of emotion. These twitching events were (unlike smiles and laughs) as frequent in RBD patients as in controls, which suggests that emotional expressions and twitches are different features and may not be generated by the same structures.

Are sleep-associated smiles and laughs mirthful?

In adults, there were several lines of evidence that smiles and laughs were not automatic but reflected true mirth. First, open mouth smiles outnumbered closed mouth smiles. Open mouth smiles in babies are associated with situations of social engagement and with high levels of arousal and positive emotional valence in the infant (Messinger et al., 2001). They are exceptional during REM sleep in new-borns, in sharp contrast with a high frequency during communicative wake (Cecchini et al., 2011). In sleeping neonate, open mouth smiles are twenty times less frequent than closed mouth smiles are (Messinger et al., 2002). Second, half of sleep-associated smiles were Duchenne smiles, which include bilateral cheek

raising and active eye closing (whether the mouth is open or not). In contrast, “non-Duchenne smiles” appear in the mouth but not the eyes and are recognized as non-genuine, often polite smiles. Duchenne smiles have been proposed as spontaneous and genuine expressions of positive emotions, such as happiness, pleasure and enjoyment (Duchenne de Boulogne, 1862-1990). Numerous studies support the association between mirth and Duchenne smiles. They selectively appear when subjects report positive subjective experiences (Ekman et al., 1990), when they watch a funny movie and “get the joke” (Juckel et al., 2011), and in association with the specific cerebral asymmetry (increased left temporal EEG activity) which characterizes happiness (Ekman et al., 1990). People producing Duchenne smiles are rated more positively (i.e., authentic, genuine, real, attractive, trustworthy) (Gunnery and Ruben, 2016), and while Duchenne smiling they increase psychological, social and spatial proximity, whereas a non-Duchenne smile is associated with psychological distance (Bogodistov and Dost, 2017). In contrast to open mouth smiles, which are rare during new-born sleep, new-borns display Duchenne smiles (Messinger et al., 2002). It is tempting to associate Duchenne smiles, as well as open mouth smiles and laughs during sleep with true mirth, as they are strongly associated during wakefulness. However, a definitive proof of this association (as in new-borns) is lacking. Non-Duchenne smiles, which represented one fourth of “happy” faces during sleep here, are more ambiguous in terms of emotional valence as people smiling awake may be happy (with a lower intensity than when displaying Duchenne smiles), but they may also try to hide embarrassment, uncertainty, or sadness.

Laughs were abundant during sleep too (N = 79) and were associated with shrugging of shoulders, peals of laughter or concomitant joyful utterances, all suggestive of true concomitant happiness. In addition, half of the smiles and laughs were temporally synchronized with REMs in REM sleep. Because REMs are associated with activation of the amygdala (the brain structure activated during awake emotions) in normal REM sleep (Calvo and Fernández-Guardiola, 1984, Miyauchi et al., 2009, Andrillon et al., 2015, Corsi-Cabrera et al., 2016), one may infer that many happy emotional expressions are associated with amygdala activation. Happy expressions were not associated with increased heart rate variability during sleep, as is sometimes observed when smiling while awake

(Ekman et al., 1983). However, most of the smiles and laughs were displayed by patients with RBD. In patients with RBD, autonomic control is altered during REM sleep (Pyatigorskaya et al., 2016), to the point that heart rate is not increased even during violent and active behaviours. The dysautonomia may blunt heart rate and vessel changes during a happy emotion. In addition, even in normal subjects, the autonomic system is modified during REM sleep, including selective reduction in somatic and sympathetic tone, which may block outward manifestations (e.g., tachycardia, sweating) of the emotional experience. Consequently, it cannot be determined whether the lack of change in heart rate during REM sleep smile is caused by absent emotion or by absent autonomic outward signs of these emotions. During NREM sleep, happy facial expressions were unfortunately too rare to conduct proper statistical analysis on heart rate variability.

We did not collect the dream/emotion recall immediately after laughs and smiles upon awakening, as this was an observational (not an interventional) study and as we did not want to disturb sleep. However, we collected the morning dream recalls, but, probably because they were collected several hours after the smiling/laughing behaviour, pleasant recalls were similarly frequent after smiling and non-smiling nights. However, on two occasions, we had the chance to observe scenic behaviour including smiles and laughs, in NREM and in REM sleep; both behaviours were highly suggestive of a concomitant pleasant scenario (the pleasure of eating a cake, and a scenario including a kiss). Conversely, there was no smiling or laughing during visibly unpleasant scenic behaviours (including fights, fracases, and fear shouting). All in all, several elements, although there are not definitive, suggest that many smiles and laughs reflect a pleasant concomitant inner emotion.

Limitations

Our study has several limitations. First, there were only 18 healthy subjects in the non-parasomniac group (having slept one or two nights in the laboratory), which makes the estimate of the percentage of spontaneous smiles and laughs asleep in normal adult sleep less certain here than if 100 healthy controls had been monitored. Other non-parasomniac subjects included patients with sleep disorders

(insomnia, hypersomnia) that are normally not associated with abnormal movements during sleep, but also patients with narcolepsy and with Parkinson's disease, who had no RBD at time of this study, but may later develop some RBD. Moreover, even this small sample of healthy volunteers was sufficient to find smiles and laughs while asleep. The sleepwalking group was less than half the size of the RBD group, which may have limited the ability to show difference of prevalence of smiling expression between these two groups. Second, the scoring of happy emotional expressions did not follow the most frequently used Facial Activity Coding System, but that system was developed for awake subjects with their eyes open. Instead, we chose to use the scoring system developed for scoring smiles and laughs in sleeping neonates and babies (who have closed eyes). Another limitation is that most observations were performed in patients with RBD, as they had the highest number of happy facial expressions. On the other hand, the patients with RBD, as well as those with sleepwalking, provide a unique opportunity to study concomitant behaviours and try to determine whether happy facial expressions occur in the context of a visibly pleasant scenario.

Future research into the connection between the felt emotion and its emotional expression may be performed by collecting the recalled emotion, having awakened the subject in the seconds after she smiled while asleep. Additionally, one may use brain functional imaging in sleeping, video-monitored subjects, and look for neural correlates of their happy emotional expressions. Both experiences are complex and challenging because REM sleep is difficult to reach when sleeping in a functional magnetic resonance imaging system and because smiles and laughs are rare, unpredictable events in adult sleep.

CONCLUSIONS

Smiling and laughing (which is particular to humans) occasionally persist during adult sleep. There are several lines of evidence that these happy emotional expressions may reflect a true inner mirth. The high frequency of happy emotional expression during RBD and its putative isomorphism suggests that the RBD model could be a small but direct window into the emotional component of REM sleep

dreams and could be used in the future to better understand the mood regulatory function and emotion desensitization during sleep.

Conflict of interest

All authors declare that they have no conflicts of interest related to this study.

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AUTHORS' CONTRIBUTION

Participants were included by MC, who also placed the EMG electrodes and performed the Ekman test. Faces and muscles were evaluated by MC and doubled-controlled by JBM. Autonomic changes were measured by JL. Data were collected by MC and JBM. The statistical analysis was performed by JBM. MC and IA drafted the first manuscript, which was amended by all co-authors. IA and MV obtained the funding.

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Table 1 - Clinical measures in non-parasomniac subjects, patients with sleepwalking and patients with REM sleep behaviour disorder

Groups	Non-parasomniac	**Sleepwalking**	**RBD**	**P***
Subjects, No.	100	22	52	NA
Recorded nights, No.	121	38	71	NA
Sex ratio, male %	53/47 (53)	10/12 (45.5)	31/21 (59.6)	0.5
Age, y	49.8 ± 19.6[a,b]	33.3 ± 9[c]	59.7 ± 15.2	<0.0001
Epworth sleepiness score, 0-24	10.5 ± 5.5	9.2 ± 4.4	9.7 ± 5.4	0.01
Ekman 35 faces test				
Total score, 0-35	28.2 ± 3.8	29.4 ± 2.9	28.1 ± 3.5	0.62
Neutral, 0-5	4.8 ± 0.7	4.9 ± 0.3	4.8 ± 0.6	0.79
Sadness, 0-5	3.1 ± 1.2	3.2 ± 1.3	3.2 ± 1.3	0.61
Happiness, 0-5	4.9 ± 0.3	4.9 ± 0.3	5 ± 0.2	0.29
Anger, 0-5	4.1 ± 1.3	4.5 ± 1.1	3.9 ± 1.1	0.78
Disgust, 0-5	3.9 ± 1.2	4 ± 0.3	3.9 ± 0.9	0.84
Fear, 0-5	3 ± 1.3	3.3 ± 1.5	2.8 ± 1.2	0.33
Surprise, 0-5	4.4 ± 1	4.8 ± 0.5	4.5 ± 0.9	0.36

Data are expressed as the mean ± SD or %. * Analyze of variance between the three groups. Post hoc test: $p<0.01$ for a difference between [a]non parasomnia and sleepwalking, [b]non parasomnia and RBD, and [c]sleepwalking and RBD groups. Tests are adjusted for age. RBD = REM sleep behaviour disorder.

Table 2 - Type and frequency of smiles and laughs during sleep in subjects

Group	Non parasomniac	Sleepwalking	RBD	P
No of subjects	100	22	52	NA
Smiles (E2 + E3 types), N (%) of subjects with				
Sleep	8 (8)	2 (9)	27 (51.9)[b,c]	<0.0001
NREM sleep	1 (1)	1 (4.5)	7 (13.5)[b]	0.004
REM sleep	7 (7)	1 (4.5)	20 (38.5)[b,c]	<0.0001
Laughs (E4), N (**%) of subjects with**				
Total sleep time	0 (0)	1 (4.5)	18 (39.6)[b]	<0.0001
NREM sleep	0 (0)	1 (4.5)	0 (0)	NA
REM sleep	0 (0)	1 (4.5)	18 (39.6)[b]	<0.0001
Duchenne smiles, N (**%) of subjects with**				
Total sleep time	2 (2)	1 (4.5)	28 (53.8)[b,c]	<0.0001
NREM sleep	1 (1)	1 (4.5)	4 (7.7)	0.15
REM sleep	1 (1)	1 (4.5)	24 (46.1)[b,c]	<0.0001
All happy emotional expressions (E2, E3, E4), N (**%) of subjects with**				
Total sleep time	8 (8)	3 (13.5)	30 (57.7)[b,c]	<0.0001
NREM sleep	1 (1)	2 (9)	10 (19.2)[b]	0.0003
REM sleep	7 (7)	2 (9)	20 (38.5)[b]	<0.0001

Post hoc test: $p<0.01$ for a difference between [a]non-parasomnia and sleepwalking, [b]non-parasomnia and RBD, and [c]sleepwalking and RBD.

Table 3 – Number and frequency of laughs and smiles during sleep in subjects with and without parasomnia**.**

Group	Non parasomniac	Sleepwalking	RBD	P
Subjects, N	100	22	52	NA
Nights, N	122	38	75	NA
All types of lip raising (unilateral lip corner raising, mild smile, open mouth smile, laugh), total number for the group; n/h of sleep				
Total sleep time	19; 0.023 ± 0.071	17; 0.072 ± 0.238	182; 0.382 ± 0.503	<0.0001
NREM sleep	7; 0.011 ± 0.053	14; 0.071 ± 0.273	21; 0.059 ± 0.132	NS
N1	0; 0 ± 0	0; 0 ± 0	3; 0.538 ± 3.614	NA
N2	6; 0.017 ± 0.086	12; 0.095 ± 0.36	16; 0.078 ± 0.213	NS
N3	1; 0.004 ± 0.044	2; 0.033 ± 0.142	2; 0.012 ± 0.075	NS
REM sleep	12; 0.13 ± 0.615	3; 0.062 ± 0.225	161; 1.863 ± 2.811[b,c]	<0.0001
Unilateral lip corner raising (E1), total number for the group; n/h of sleep				
Total sleep time	10; 0.013 ± 0.063	13; 0.056 ± 0.224	22; 0.051 ± 0.1	NS
NREM sleep	6; 0.01 ± 0.052	12; 0.061 ± 0.25	12; 0.036 ± 0.1	NS
N1	0; 0 ± 0	0; 0 ± 0	2; 0.116 ± 0.7	NA
N2	5; 0.15 ± 0.082	11; 0.09 ± 0.4	8; 0.042 ± 0.15	NS
N3	1; 0.004 ± 0;044	1; 0.016 ± 0.1	2; 0.012 ± 0.08	NS
REM sleep	4; 0.028 ± 0.155	1; 0.026 ± 0.16	10; 0.109 ± 0.34	NS
Mild smile (E2), total number for the group; n/h of sleep				
Total sleep time	6; 0.006 ± 0.029	1; 0.003 ± 0.02	36; 0.077 ± 0.156 [b,c]	<0.0001
NREM sleep	1; 0.001 ± 0.013	1; 0.004 ± 0.026	7; 0.018 ± 0.061	NS
N1	0; 0 ± 0	0; 0 ± 0	0; 0 ± 0	NA
N2	1; 0.002 ± 0.026	1; 0.005 ± 0.031	7; 0.031 ± 0.111	NS
N3	0; 0 ± 0	0; 0 ± 0	0; 0 ± 0	NA
REM sleep	5; 0.075 ± 0.569	0; 0 ± 0	29; 0.389 ± 0.911	<0.0001

Group	Non parasomniac	Sleepwalking	RBD	P
Open-mouth smile (E3), total number for the group; n/h of sleep				
Total sleep time	3; 0.003 ± 0.019	1; 0.003 ± 0.019	47; 0.1 ± 0.195 [b,c]	<0.0001
NREM sleep	0; 0 ± 0	0; 0 ± 0	2; 0.006 ± 0.035	NA
N1	0; 0 ± 0	0; 0 ± 0	1; 0.423 ± 3.56	NA
N2	0; 0 ± 0	0; 0 ± 0	1; 0.005 ± 0.039	NA
N3	0; 0 ± 0	0; 0 ± 0	0; 0 ± 0	NA
REM sleep	3; 0.027 ± 0.193	1; 0.012 ± 0.074	45; 0.543 ± 1.131 [b,c]	<0.0001
Laughs (E4), total number for the group; n/h of sleep				
Total sleep time	0; 0 ± 0	2; 0.01 ± 0.043	77; 0.153 ± 0.306 [b,c]	<0.0001
NREM sleep	0; 0 ± 0	1; 0.006 ± 0.037	0; 0 ± 0	NA
N1	0; 0 ± 0	0; 0 ± 0	0; 0 ± 0	NA
N2	0; 0 ± 0	0; 0 ± 0	0; 0 ± 0	NA
N3	0; 0 ± 0	1; 0.017 ± 0.106	0; 0 ± 0	NA
REM sleep	0; 0 ± 0	1; 0.024 ± 0.15	77; 0.822 ± 1.741[b,c]	<0.0001
Duchenne smiles*, total number for the group; n/h of sleep				
Total sleep time	2; 0.003 ± 0.023	3; 0.013 ± 0.046	103; 0.304 ± 0.724[b,c]	<0.0001
N2 sleep	1; 0.003 ± 0.034	0; 0 ± 0	5; 0.027 ± 0.134	NA
N3 sleep	0; 0 ± 0	1; 0.017 ± 0.106	0; 0 ±0	NA
REM sleep	1; 0.07 ± 0.08	2; 0. 036 ± 0.164	98; 0.993 ± 1.99 [b,c]	<0.0001
All happy emotional expressions (E2, E3, E4), total number for the group; n/h of sleep				
Total sleep time	9; 0.009 ± 0.036	4; 0.016 ± 0.049	160; 0.331 ± 0.498[b,c]	<0.0001
NREM sleep	1; 0.001 ± 0.013	2; 0.01 ± 0.044	9; 0.023 ± 0.075[b]	<0.0001
N1	0; 0 ± 0	0; 0 ± 0	1; 0.423 ± 3.56	NA
N2	1; 0.002 ± 0.026	1; 0.005 ± 0.031	8; 0.036 ± 0.129	NA
N3	0; 0 ± 0	1; 0.017 ± 0.106	0; 0 ± 0	NA
REM sleep	8; 0.102 ± 0.6	2; 0.036 ± 0.165	151; 1.754 ± 2.806[b,c]	<0.0001

Group	Non parasomniac	Sleepwalking	RBD	P
Pleasant dream recall, N (%)	9 (7.3)	5 (13.1)	4 (5.3)	0.33

Results are expressed as numbers or means ± SD. See the Results section for statistics. NA = not applicable (small samples). NS: not significant. Post hoc test: $p<0.01$ for a difference between [b]non-parasomnia and RBD, and [c]sleepwalking and RBD groups. * Duchenne smiles occured in mild (E2) or in open mouth (E3) smiles.

Figure legends

Figure 1 - Examples from real patients (redrawn to prevent face recognition) illustrating the coding system for the facial expressions.

Figure 2 - Illustrations (redrawn from real patients) of smiles and laughs observed during NREM and REM sleep in healthy controls, in patients with sleepwalking (not during sleepwalking episodes) and in patients with REM sleep behaviour disorder (RBD).

Supplemental Figure - Change in heart rate before, during (starting at 0 s) and after smiling during sleep. For each subject, the baseline value is the heart rate measured 5 s before starting to smile.

Legends for the video clips

Video clip #1 – Patient with sleepwalking, having a parasomniac event arising from N3 sleep, suggesting a happy scenario: “Eh …! T’as fait du cake.. C’est bon ça c’est bon. Mets le gateau dans la basket; demain matin il sera très très bon, “perfetto”… Il y a des candidats là ?” (*unintelligible words*) *Laughs* “Oh, punaise!” (English translation: Eh! but what…? You made some cake, that’s good, that, that’s good. Put the cake in the basket there. Tomorrow, it will be very, very good – perfect – ... Hey are there some candidates there? (*unintelligible words*) *Laughs*, “Oh boy!”.) (Shown with the permission of the patient).

Video-clip #2 - Patient with a mild form of REM sleep behaviour disorder associated with Parkinson’s disease, laughing during REM sleep (Shown with the permission of the patient). She later reported that she dreamt of attending a happy wedding party.

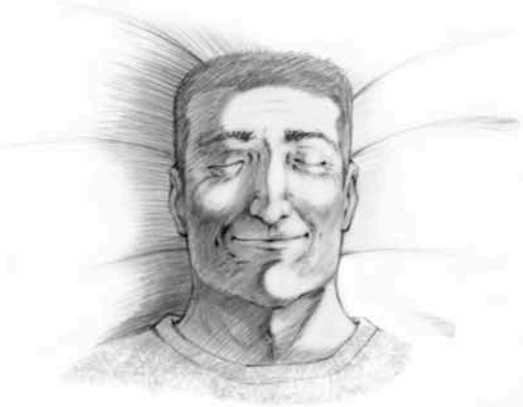
0 = Neutral

1 = Isolated lip corner raising

2 = Mild smile

3 = Open-mouth smile

4 = Laugh



Healthy control in N2 sleep

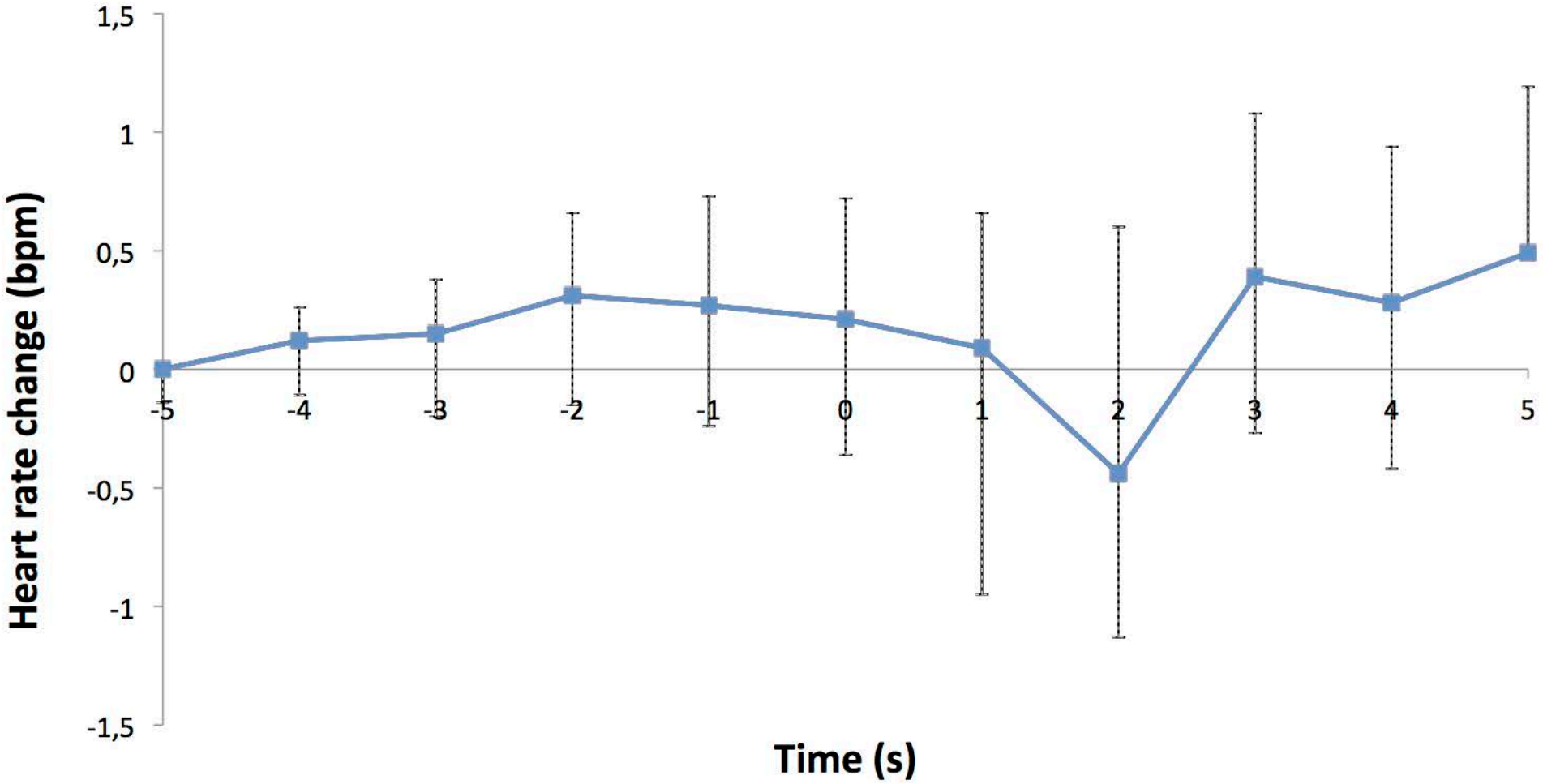
Healthy control in REM sleep

Patient with sleepwalking, in N2 sleep

Patient with sleepwalking, in REM sleep

Patient with RBD, in N2 sleep

Patient with RBD, in REM sleep


1,5
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-1
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-3
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1
2
3
4
5
Heart rate change (bpm)
Time (s)