ZOOLOGICAL RESEARCH

Potential use of actigraphy to measure sleep in monkeys: comparison with behavioral analysis from videography

DEAR EDITOR,

Since the epoch-making observations of circadian rhythm in Mimosaceae plants, sleep has been investigated for centuries (de Mairan, 1729; Du Monceau, 1758). As a natural and reversible state, sleep is marked by reduced responsiveness to external stimuli, relative inactivity, and loss of consciousness. Although reduced responsiveness could potentially introduce significant danger to survival, nearly all animals in nature sleep. This strongly implies an adaptive role of sleep in increasing overall fitness of an organism. Research has shown that sleep is responsible for many vital physiological functions, including tissue repair (Oswald, 1980), skin function (Rechtschaffen, 1998), thermoregulation (Parmeggiani, 1986; Rechtschaffen, 1998), energy saving (Siegel, 2005), insulin release and responsiveness (Spiegel et al., 1999), metabolic regulation (Sharma & Kavuru, 2010), immunological enhancement (Besedovsky et al., 2012; Imeri & Opp, 2009), synaptic plasticity (Benington & Frank, 2003), neuron viability (Zhang et al., 2014a), and memory formation (Rasch & Born, 2013; Walker & Stickgold, 2006). Sleep therefore plays an essential role in human health and is vital for physical and psychological performance (Halson & Juliff, 2017; Thun et al., 2015; Vitale & Weydahl, 2017).

Given its indispensable functions, insufficiency in sleep can cause a cascade of negative consequences for general health, as well as cardiovascular, metabolic, mental, and immunological health, and in cancer, pain, and all-cause mortality (Parekh et al., 2015; Scullin & Bliwise, 2015; Vgontzas et al., 2013; Watson et al., 2015a, 2015b, 2015c, 2015d). Sleep is also equally important in behavioral and physical performance. For example, sleep deprivation and extension are negatively and positively associated with athletic performance, respectively (Thun et al., 2015), suggesting that chronotype effects should be considered during the scheduling of training sessions (Vitale & Weydahl, 2017). According to the third edition of the International Classification of Sleep Disorders (ICSD), there are seven major sleep disorders, including insomnia, sleep-related breathing disorders, central disorders of hypersomnolence, circadian rhythm sleep-wake disorders, sleep-related movement disorders, parasomnias, and other sleep disorders (Sateia, 2014).

Although human subjects are an ideal choice for studies on sleep and sleep disorders, animal-based research has played a fundamental role in the elucidation of the mechanisms that underlie sleep, as well as its regulation and disorders, and is essential for validating sleep mechanisms and testing therapeutics for sleep disorders (Singh et al., 2017; Toth & Bhargava, 2013). Sleep deprivation experiments have been successfully performed in rats (Rechtschaffen et al., 1989), mice (Mackiewicz et al., 2007; Maret et al., 2007), fruit-flies (Shaw et al., 2002), and roundworms (Sanders et al., 2017). Non-human primates (NHPs) are among the best-studied animal models, in large part because of their close phylogenetic relatedness to humans (Nunn & Samson, 2018; Zhang et al., 2014b). Likewise, sleep also plays a dominant role in NHP health, behavior, and ecology, and can exert a significant influence on daily activity schedules (Anderson, 1998, 2000; Qiu et al., 2019). Thus, NHPs are especially valuable for comparative studies on sleep, with tremendous potential to provide critical improvement in our understanding of human sleep and associated disorders (Fruth et al., 2018; Nunn et al., 2010).

Electroencephalography (EEG) is commonly used in sleep

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research and can provide an objective and functional marker of sleep (Feinberg et al., 1967; Roffwarg et al., 1964). Although EEG can be performed in restrained or freely moving animals under controlled laboratory conditions, this technique is invasive, involving implantation of electrodes in the brain or subcutaneously. Implantation usually involves drilling holes in the skull to place electrodes directly on the brain. Compared with a single-channel EEG, polysomnogram (PSG) is considered the gold standard to objectively assess sleep (Boulos et al., 2019; O'Donnell et al., 2018). PSG integrates both normal and abnormal physiological indicators during sleep, including brain activity (EEG), eye movements (EOG: electrooculography), muscle activity (EMG: electromyography), heart rhythm, respiratory effort, airflow through the mouth and nose, and audible snoring. However, a significant limitation of PSG is that it requires electrodes attached to the scalp and skin surface, and sensors to collect data (Lucey et al., 2016). These are difficult or impossible to apply in freely moving monkeys. Even though subcutaneous implantations are minimally invasive, PSG is expensive and burdensome to obtain and may be difficult to use with longer recording intervals. This greatly limits the use of PSG or EEG, especially for long-term research involving a large sample size.

The emergence of videography has offered convenience for observing behaviors in naturally sleeping monkeys (Chen et al., 2017; Kripke et al., 1968; Weitzman et al., 1965). The video technique avoids the need for surgery and electrode implantation, and most importantly, it is inexpensive and noninvasive, allowing for large sample size and long-term study by means of specific behavioral criteria defined for each state. Nevertheless, manual video analysis presents some limitations, including greater subjectivity and considerable human labor and time requirements. In comparison, actigraphy provides a more objective measure than videography, and can be considered as an alternative sleep assessment method in research (Ancoli-Israel et al., 2003; Littner et al., 2003; Morgenthaler et al., 2007; Sadeh et al., 1995; Sadeh & Acebo, 2002; Thorpy et al., 1995). The use of actigraph accelerometers for detection of movement is a reliable, noninvasive method for monitoring activity (Andersen et al., 2010, 2012; Mann et al., 2005). Despite the increase in studies utilizing actigraphy, no comparisons in sleep scoring based on actigraphy and videography have been conducted in NHPs.

The present study was designed to compare videographic and actigraphic sleep scoring in 10 cynomolgus monkeys over seven nights of simultaneous behavioral recordings, and to validate the use of actigraphy in sleep measurement.

Mean locomotor activity for each monkey was measured by Actical monitors attached to the monkeys' necks (n=10). Data collected by actigraphy over the seven days were presented as means of that time period. As shown in Figure 1A, the monkeys displayed consistently high activity during light time (0800 h–2000 h) and low activity during dark time (2000 h–0800 h).

Figure 1B shows the correlations between the mean time spent in each state per night obtained from actigraphy and videography analysis. Results showed a positive correlation between the two methodologies for scoring the state of wake (r=0.368 P= 0.002) and sleep (r=0.368, P= 0.002). When measuring the state of transitional sleep (r=–0.058, P=0.631) and relaxed sleep (r=0.174, P= 0.149), no significant correlation was observed.

Figure 1C shows the differences and limits of agreement between actigraphy and videography measurements. Results indicated that, compared with videographic analysis, actigraphy underestimated the durations of wake and transitional sleep by an average of 124.929 min and 132.271 min, respectively, but overestimated the durations of sleep and relaxed sleep by 124.929 min and 257.200 min, respectively.

Total durations of time spent in each state for each monkey based on videography and actigraphy are illustrated in Supplementary Table S1. When comparing the differences for each state between the two methods, the durations of time spent in each state obtained from actigraphy were significantly different from those obtained by videography (wake: actigraphy vs. videography=16.629±1.404 vs. 141.557±4.652 min per night, P=1.771×10^{-54} ; sleep: actigraphy vs. videography=703.371±1.404 vs. 578.443±4.652 min per night, P=1.771×10^{-54}; transitional sleep: actigraphy vs. videography=67.157±2.915 vs. 199.429±6.009 min per night, P=3.626×10^{-42}; relaxed sleep: actigraphy vs. videography=636.214±3.889 vs. 379.014±8.706 min per night, P=7.009×10^{-57}).

The epoch-by-epoch analysis results are presented in Supplementary Table S2 and Table 1 and relate to the duration of epochs and percentages for all dark period recordings in all monkeys. The maximum percentage agreement between the two methods relative to the state of sleep reached 99.852%. Of the 40 491 epochs scored for sleep by videography, 40 431 (99.852%) were correctly scored by actigraphy analysis and 60 (0.148%) were allocated to the state of wake. Of the 9 909 wake epochs, however, only 1 104 (11.141%) were correctly scored by actigraphy, with 8 805 epochs (88.859%) scored as sleep. This demonstrates that actigraphic analysis can correctly score the state of sleep but may not correctly score the state of wakefulness.

To further determine whether actigraphy is suitable for scoring states of sleep. As shown in Table 1, actigraphic analysis was preferable for scoring the state of relaxed sleep. Of the 26 531 relaxed sleep epochs, 25 886 (97.569%) were correctly scored using actigraphy, with 34 (0.128%) and 611 epochs (2.303%) respectively scored as the state of wake and transitional sleep. Actigraphy also correctly scored 800 epochs (5.731%) as transitional sleep out of the 13 960 epochs of transitional sleep obtained from videographic analysis. Of the remaining inconsistently scored transitional sleep epochs, 13 134 (94.083%) were allocated to relaxed sleep and 26 (0.186%) were allocated to wakefulness.

In the present study, actigraphy constituted a reliable

method for scoring the state of sleep in monkeys (Andersen et al., 2013; Berro et al., 2016; Golub & Hogrefe, 2016; Kantha & Suzuki, 2006), showing a significant correlation in comparison with states obtained by videography. Epoch-by-epoch analysis provided an exact measure of the percentage agreement between the two methods, which showed that actigraphy could accurately score the state of sleep (99.852%). This is consistent with results previously obtained in humans, with actigraphy demonstrating 93% and 94% sensitivity in measuring sleep compared with PSG (Niel et al., 2019; Yavuz-Kodat et al., 2019). In four species of lemur, Cramer's V correlation between actigraphy-classified sleep and videography-classified sleep revealed highly consistent results (Melvin et al., 2019). Here, although the sensitivity of actigraphy in detecting sleep was very high, it performed poorly in detecting wakefulness, with only 11.141% of epochs

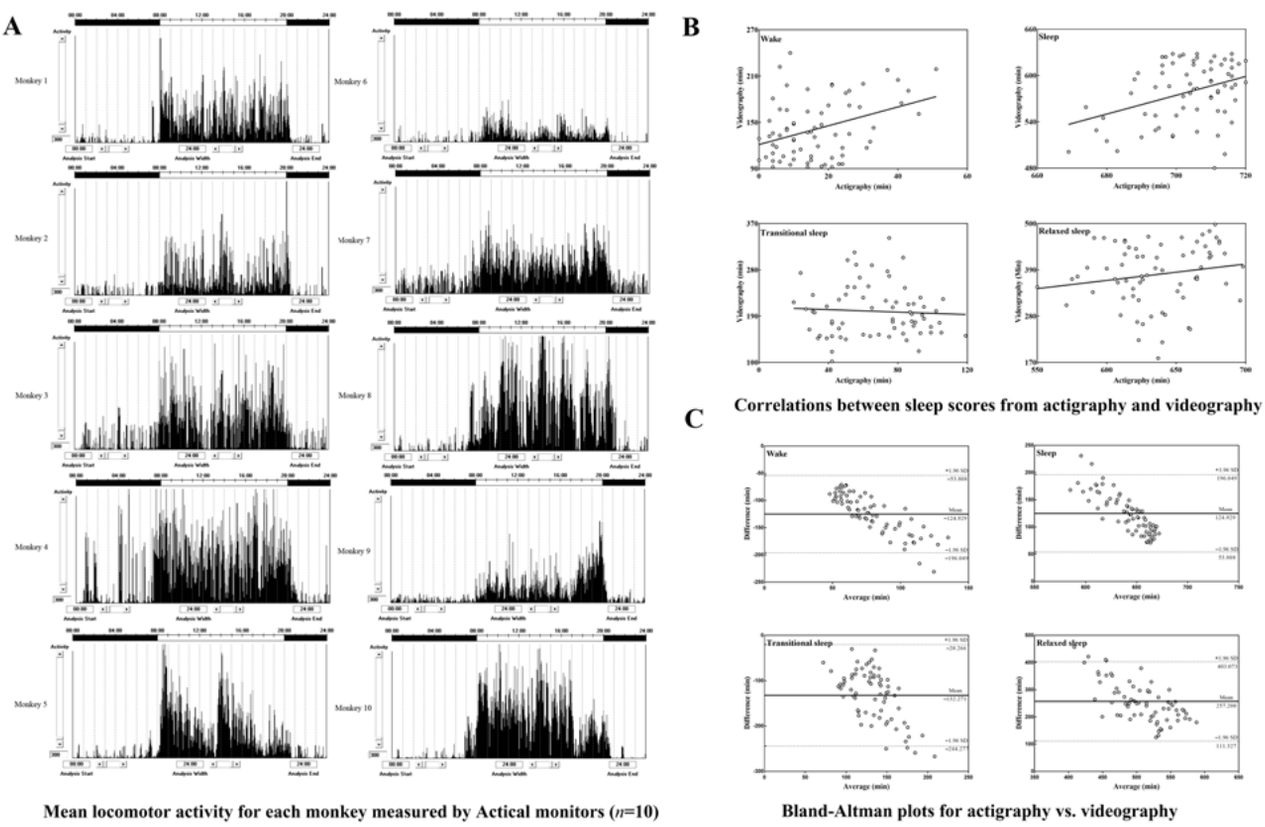

Figure 1 Locomotor activity, correlations, and Bland-Altman plots

A: Mean locomotor activity for each monkey measured by Actical monitors (*n*=10). Activity data were collected over a seven-day period and are presented as means over that period. Lights were on at 0800 h and off at 2000 h. B: Correlations between sleep scores from actigraphy and videography. Four states were scored by actigraphy (X-axis: Actigraphy) and videography analysis (Y-axis: Videography), respectively, including wake, sleep, transitional sleep, and relaxed sleep. C: Bland-Altman plots for actigraphy vs. videography. X axes represent average of two methods ((actigraphy+videography)/2) and Y axes show differences between two paired measurements (actigraphy–videography), including wake, sleep, transitional sleep, and relaxed sleep. Solid lines show mean differences between actigraphy and videography measurements; dotted lines represent 95% limits of agreement, from –1.96 *SD* (standard deviation) to +1.96 *SD*.

Table 1 Epoch-by-epoch analysis for each state (including wake, transitional sleep, and relaxed sleep) showing total duration (min) and corresponding percentage (%) of epochs scored in agreement (diagonal from left to right) between videography (rows) and actigraphy (columns) methods and those scored differently by actigraphy analysis

State	Min			%		
	Wake	Transitional sleep	Relaxed sleep	Wake	Transitional sleep	Relaxed sleep
Wake	1 104	3 290	5 515	11.141	33.202	55.656
Transitional sleep	26	800	13 134	0.186	5.731	94.083
Relaxed sleep	34	611	25 886	0.128	2.303	97.569

correctly identified. This accords with previous research comparing actigraphy and polysomnography in older adults (Sivertsen et al., 2006). These results suggest that actigraphy may show poor sensitivity in detecting wakefulness during the sleep-period (Terrill et al., 2010). This may be because the monitors were mounted to the monkeys' necks, and small movements may not be detected, such as hand movements involving no changes of position. As a result, 8 805 epochs (88.859%) were wrongly scored as the state of sleep.

Further epoch-by-epoch analysis indicated that actigraphy was more suitable for scoring the state of relaxed sleep, with 97.569% of relaxed sleep (25 866 epochs) correctly identified in comparison with videography. Only 34 (0.128%) and 611 epochs (2.303%) were differently interpreted as wake and transitional sleep, respectively, compared with videographic analysis. One of the most important reasons for this result is that relaxed sleep was scored when the monkeys exhibited neither body nor limb movements and the activity monitor was highly sensitive to detecting the state of rest. When the monitor did not detect any movements within 1 min, this minute was scored as relaxed sleep. In contrast, the actigraphy method could not easily discriminate between the state of relaxed and transitional sleep (800 epochs, i.e., 5.731%). Although a monkey may have exhibited one or two movements within 1 min, the monitor could not detect such movement, and thus, 94.083% of transitional sleep (13 134 epochs) was incorrectly allocated to the state of relaxed sleep. Also, a tiny fraction of transitional sleep was interpreted as wakefulness (26 epochs, i.e., 0.186%).

In view of the differences between the two sleep scoring methods, compared with videographic analysis, actigraphy underestimated the durations of wake and transitional sleep by 88.253% and 66.325%, respectively, but overestimated the durations of sleep and relaxed sleep by 21.597% and 67.860%, respectively. One reason for this is because the neck-attached monitors showed poor sensitivity to slight movements by the monkeys. Most of the time, the monitors could not detect any movements, and thus interpreted the state of wakefulness (≥3 movements within 1 min) and transitional sleep (1–2 movements in 1 min) as either sleep or relaxed sleep. A wrist-attached monitor would likely provide more accurate results and is a commonly used site for sleep monitoring (Mathie et al., 2004). Periods of sleep are usually accompanied by minimal movement, with relatively more movements occurring during the periods of wakefulness, which can be better discriminated by wrist actigraphy (Slater et al., 2015; Tryon, 1996). However, certain populations, such as children with neurodevelopmental disorders, may have difficulty tolerating wrist placement (Adkins et al., 2012). In this case, other locations have also been utilized to measure sleep, including the leg (Middelkoop et al., 1997), waist (Enomoto et al., 2009; Paavonen et al., 2002), shoulder (Adkins et al., 2012), and hip (Zinkhan et al., 2014). Although most validation studies comparing actigraphy to the gold standard measure of polysomnography have used non-dominant wrist placement, other locations can be used if the wrist is not available or subjects are unable to tolerate wrist placement. In the present study, the neck location was utilized to avoid possible adverse effects on daily routine and allow for longer-term wear. The new generation of accelerometers provides high temporal and intensity resolution, allowing the detection of tiny body movements and identification of pulse waves when attached to the wrist (Zschocke et al., 2019). Even when worn on the wrist or hip, these devices provide data that can be used to track respiratory rate throughout the night (Zinkhan & Kantelhardt, 2016). As such, these high-resolution accelerometers should detect tiny motions, even when attached to the neck, and triaxial recordings may be able to distinguish whether the monkey is in an upright position or lying down. Algorithms are especially important for the deduction of sleep measures. At present, however, most algorithms have been developed and validated for wrist measurements. When accelerometers are placed at the hip, mean PSG bias is larger, and there is a distinct dependency in differences of magnitude of respective sleep measures compared to wrist measurements (Zinkhan & Kantelhardt, 2016). Thus, new algorithms need to be developed to adapt to the different placements of accelerometers.

Although the actigraphic approach showed poor sensitivity for detecting wakefulness in the current study, it could play a supportive role in the elucidation of sleep differences between control and specific disease model monkeys. In comparison with EEG, this method does not require deep or subcutaneous implantation of electrodes, and thus is almost noninvasive, which could help reduce adverse effects on monkeys, such as infection from surgery. In addition, EEG and PSG equipment is relatively expensive and may be difficult to use under long recording intervals and large sample sizes. Although videography is noninvasive and can allow observations of behaviors in naturally sleeping monkeys (Kripke et al., 1968; Weitzman et al., 1965), it requires greater subjectivity and considerable human labor and time costs. In consideration of the above-mentioned factors and the results reported in this study, the use of actigraphy for scoring sleep (especially relaxed sleep) shows potential for future research on sleep in NHPs. The behavioral criteria and approach were validated in this study and could be considered as a complementary technique to conventional EEG and/or videography analysis for sleep studies in NHPs.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

X.T.H., L.X., Y.L., and Y.C.C. designed the study. D.D.Q. and S.F.F. performed the experiments, analyzed the data, and drafted the initial manuscript. D.D.Q., S.F.F., W.J.S., and Y.Z.

acquired the behavioral data. F.Y.Z., N.W., T.F.X., X.L.X., X.T.Y., X.Z., and X.Z. conducted the behavioral analysis. All authors read and approved the final version of the manuscript.

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