Journal of Pharmacological and Toxicological Methods 70 (2014) 86–93

Contents lists available at ScienceDirect

Journal of Pharmacological and Toxicological Methods

journal homepage: www.elsevier.com/locate/jpharmtox

Original article

Effects of amphetamine, diazepam and caffeine on polysomnography (EEG, EMG, EOG)-derived variables measured using telemetry in Cynomolgus monkeys ☆

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ARTICLE INFO

Article history:
Received 12 March 2014
Accepted 20 May 2014
Available online 27 May 2014

Keywords:
Animal model
EEG spectral analysis
Macaca fascicularis
Pharmacology
Sleep stages
Telemetry

ABSTRACT

Introduction: Medication-induced sleep disturbances are a major concern in drug development as a multitude of prescription drugs alter sleep patterns, often negatively. Polysomnography is used in clinical diagnostics but is also applicable to animal models. Rodent sleep architecture (nocturnal) differs from larger diurnal mammals, including humans, increasing the translational potential of non-rodent species to the clinic. This study aimed to characterize the response to pharmacological agents known to affect sleep structure and EEG activity in a non-human primate (*Macaca fascicularis*) using telemetry-based polysomnography. **Methods**: Animals were instrumented with telemetry transmitters for continuous electroencephalogram (EEG), electro-oculogram (EOG) and electromyogram (EMG) monitoring combined with video. EEG, EMG and EOG were monitored for 12 to 24 h to establish baseline values, followed by administration of pharmacological agents (saline, d-amphetamine, diazepam or caffeine). **Results**: Amphetamine (0.3 and 1 mg/kg, by oral administration (PO)) significantly reduced total sleep time, including the duration of both non-rapid eye movement [NREM] sleep and REM sleep. It also decreased EEG activity in low frequencies (i.e., 4–6 Hz) during wakefulness. Diazepam (2 mg/kg, PO) did not significantly alter sleep duration, but importantly reduced EEG activity in low frequencies (approximately 2–12 Hz) during wakefulness, NREM and REM sleep. Finally, caffeine (10 and 30 mg/kg, PO) decreased both NREM and REM sleep duration. In addition, spectral analysis revealed important decreases in low frequency activity (*i.e.*, 1–8 Hz) during wakefulness with a parallel increase in high frequency activity (*i.e.*, 20–50 Hz) during NREM sleep. **Discussion**: As these observations are similar to previously reported pharmacological effects in humans, results support that EEG, EOG and EMG monitoring by telemetry in Cynomolgus monkeys represents a useful non-clinical model to investigate and quantify drug-induced sleep disturbances.

1. Introduction

A number of medications, including several widely used drugs such as nicotine replacement therapy (Mills, Wu, Lockhart, Wilson, & Ebbert, 2010), oral contraceptives (Baker, Mitchell, & Driver, 2001), aspirin and ibuprofen (Murphy, Badia, Myers, Boecker, & Wright, 1994) have been shown to impact on objective and subjective sleep quality in humans. Other drugs including beta-blockers, benzodiazepines and opioids were also shown to affect sleep architecture. Indeed, beta-blockers, such as propranolol, metoprolol and pindolol increase wake after sleep onset and reduce rapid eye movement (REM) sleep (Betts & Alford, 1985). Benzodiazepines decrease sleep latency, usually defined as the time elapsed from lights off to the first minute of sleep, and increase total sleep time, but also affect the proportion of the different sleep stages, by increasing time in non-rapid eye movement (NREM) sleep stage 2 and decreasing NREM sleep stage 3 and REM sleep, therefore reducing deep sleep (Buscemi et al., 2005; Carlstedt, 2009). Opioids disturb sleep and decrease duration of NREM sleep stage 2 and REM sleep (Ramakrishnan & Scheid, 2007). The reduction in sleep efficiency, which takes into account the time spent awake during the sleep period, by drugs such as caffeine and d-amphetamine has been widely reported

☆ Funding: The research was conducted thanks to a NSERC grant (386623-2010, VM), a FRQS salary award (22210, VM), start-up funds from the CR-HSCM (VM), and CiToxLAB North America.
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http://dx.doi.org/10.1016/j.vascn.2014.05.003
1056-8719/

(Bonnet & Arand, 1992; Boutrel & Koob, 2004). In addition, most pharmacological substances used as sleep medication or to treat psychiatric disorders lead to modifications of the spectral profile of sleep and wake states (Hasan et al., 2009; Paterson, Wilson, Nutt, Hutson, & Ivarsson, 2009; Schmid et al., 2006). The European Medicines Agency (2011) confirms that multichannel polysomnography is a valuable tool that enables objective assessment of sleep architecture, including sleep latency, number of awakening and total sleep time in the context of drug development for sleep disorders. EEG spectral analysis thus needs to be used to refine the characterization of drug-induced alterations in sleep.

During pharmacological development, identification of potential effects on sleep duration and architecture as well as on EEG activity during sleep can impact on safety and consequently, on prescription rate. Reduced sleep duration (e.g., sleep deprivation) has been shown to be detrimental to human health and can contribute to diabetes (Gottlieb et al., 2005), various hormonal deficiencies (Vgontzas et al., 1999) as well as neurological disorders (Palma, Urrestarazu, & Iriarte, 2013), emphasizing the importance of managing the risk associated with changes in sleep architecture in drug development. Slow wave characteristics of deep sleep (i.e., NREM sleep stage 3) have also been hypothesized to directly mediate recovery during sleep (Tononi & Cirelli, 2006). Pre-clinical models to investigate potential adverse effects of drugs on sleep as well as assessment of the efficacy of drugs that may improve quality and duration of sleep are thus necessary (Schmid et al., 2006).

In humans, sleep and wakefulness episodes are mostly consolidated in adults (Carskadon & Dement, 2011), and sleep usually occupies a third of the nycthemeron. A night of sleep usually begins with a short period of NREM sleep stage 1 (N1), which is followed by NREM sleep stage 2 (N2) characterized by the presence of sleep spindles and K-complexes. These stages are usually followed by NREM sleep stage 3 (N3) which is mostly composed of slow waves that are indexed by EEG slow wave activity (SWA, 1–5 Hz). Episodes of REM sleep, characterized by muscle atonia and intermittent REM observable on the electro-oculogram (EOG), become increasingly longer as the night progresses. Stages N1, N2, N3 and REM generally represent 2–5%, 45–55%, 10–20% and 20–25% of total sleep time in humans, respectively. In rodents, however, sleep is polyphasic with very frequent transitions between wakefulness and sleep in addition to occurring mostly during the light period (Franken, Malafosse, & Tafti, 1998; Van Twyver, 1969). Moreover, characterization of the sleep/wake cycle in mice includes wakefulness, NREM sleep and REM sleep, with NREM sleep representing close to 90% of total sleep time (C57BL/6J strain; Franken et al., 1998). As such, the mouse model lacks the different NREM sleep stages identified in humans, which may reduce sensitivity in some cases. Rodent models thus present some limitations when investigating potential drug effects on sleep duration or architecture. Conversely, Cynomolgus monkeys are diurnal and show similar proportion of sleep stages in comparison to humans (Porsolt, 2013; Rachalski et al., in press).

The fact that Cynomolgus monkeys are diurnal mammals like humans emphasizes the appropriateness of this non-human primate as a translational model of human sleep because it suggests a similar regulation of sleep by the circadian timing system. The circadian system, controlled by the suprachiasmatic nuclei (SCN) of the hypothalamus, regulates many physiological functions including the sleep-wake cycle, hormonal secretion and metabolism (Kriegsfeld, LeSauter, Hamada, Pitts, & Silver, 2002; Kriegsfled & Silver, 2006). In addition, circadian control of hormone secretion can influence sleep, which is particularly true for melatonin that also directly influences SCN activity (Kriegsfled & Silver, 2006). Similar to humans, diurnal non-human primates have elevated melatonin production during the main rest period (i.e., the dark phase), and melatonin administration favors sleep (Zhdanova et al., 2002) thus supporting their translational potential.

Scarce data are available on sleep architecture in macaque monkeys (Barraud et al., 2009; Hsieh, Robinson, & Fuller, 2008). Prolonged physical restraint during EEG monitoring (Pigarev, Almirall, & Pigareva, 2008), along with limited length of recording sessions characterizes many of the data sets previously published (Benca, Obermeyer, Shelton, Droster, & Kalin, 2000). The use of telemetry EEG monitoring enables animals to freely move allowing for the assessment of the animal's natural sleep architecture (Hsieh et al., 2008; Rachalski et al., in press). Accordingly, the current study aimed to validate the use of telemetry to investigate the effects of different pharmacological treatments on polysomnography (EEG, EOG and EMG)-derived variables, including EEG spectral activity, in unrestrained Cynomolgus monkeys.

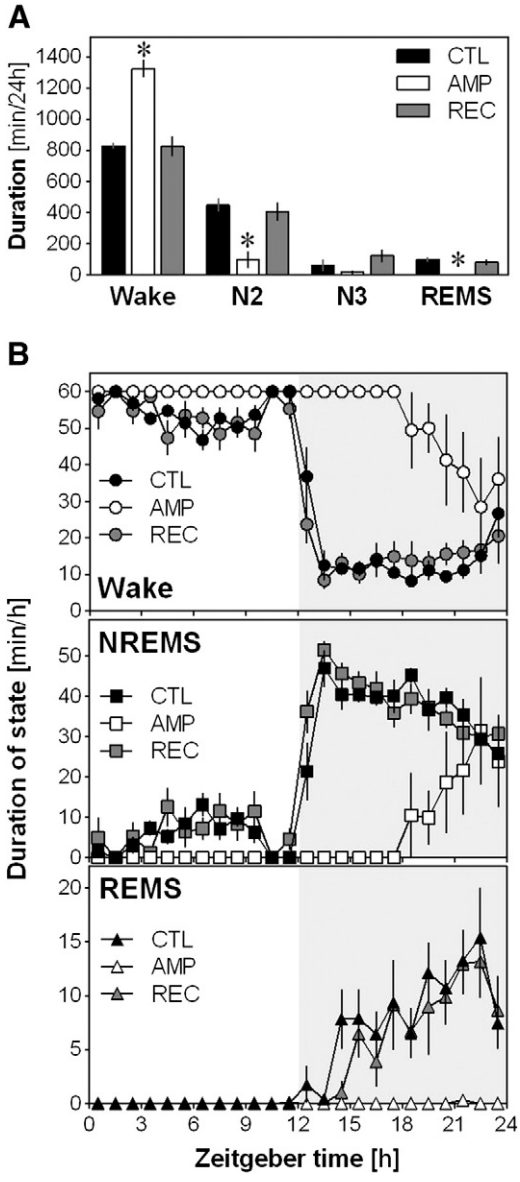

Fig. 1. Duration and distribution of vigilance states in Cynomolgus monkeys under control treatment, d-amphetamine (1 + 0.56 mg/kg, 9 h apart), and recovery. A) Total duration of wakefulness (wake), N2, N3 and REM sleep (REMS) for a 24-hour period averaged for 4 monkeys. D-amphetamine significantly changed wakefulness, N2 and REMS ($F_{2,6} > 23.3$, $p < 0.01$). Stars indicate Tukey HSD <0.01 compared to both control and recovery. B) Hourly distribution of wakefulness, NREM sleep (NREMS: N1 to N3) and REMS in control condition, under d-amphetamine (1 + 0.56 mg/kg, 9 h apart) and during recovery in 4 animals. Zeitgeber time refers to the time relative to the light/dark schedule with 0 corresponding to lights ON and 12 to lights OFF. Grey backgrounds indicate the dark period.