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Research paper

Electrophysiological characterization of sleep/wake, activity and the response to caffeine in adult cynomolgus macaques☆

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ABSTRACT

Most preclinical sleep studies are conducted in nocturnal rodents that have fragmented sleep in comparison to humans who are primarily diurnal, typically with a consolidated sleep period. Consequently, we sought to define basal sleep characteristics, sleep/wake architecture and electroencephalographic (EEG) activity in a diurnal non-human primate (NHP) to evaluate the utility of this species for pharmacological manipulation of the sleep/wake cycle. Adult, 9–11 y.o. male cynomolgus macaques (n = 6) were implanted with telemetry transmitters to record EEG and electromyogram (EMG) activity and Acticals to assess locomotor activity under baseline conditions and following injections either with vehicle or the caffeine (CAF; 10 mg/kg, i.m.) prior to the 12 h dark phase. EEG/EMG recordings (12–36 h in duration) were analyzed for sleep/wake states and EEG spectral composition. Macaques exhibited a sleep state distribution and architecture similar to previous NHP and human sleep studies. Acute administration of CAF prior to light offset enhanced wakefulness nearly 4-fold during the dark phase with consequent reductions in both NREM and REM sleep, decreased slow wave activity during wakefulness, and increased higher EEG frequency activity during NREM sleep. Despite the large increase in wakefulness and profound reduction in sleep during the dark phase, no sleep rebound was observed during the 24 h light and dark phases following caffeine administration. Cynomolgus macaques show sleep characteristics, EEG spectral structure, and respond to CAF in a similar manner to humans. Consequently, monitoring EEG/EMG by telemetry in this species may be useful both for basic sleep/wake studies and for pre-clinical assessments of drug-induced effects on sleep/wake.

1. Introduction

The cyclic alternation between wakefulness and sleep appears to be ubiquitous among mammals (Siegel, 2005; Lesku et al., 2008) and, over the last several decades, has also been recognized to occur in other vertebrates (Beckers and Rattenborg, 2015; Shein-Idelson et al., 2016; Oikonomou and Prober, 2017) as well as invertebrates including molluscs (Vorster et al., 2014), flies (Artiushin and Sehgal, 2017), nematodes (Trojanowski and Raizen, 2016) and ctenophores (Nath et al., 2017). Among mammals, the sleep/wake patterns of non-human primates (NHPs) more closely resemble the consolidated periods of sleep and wakefulness of humans than do the polyphasic sleep/wake cycles of nocturnal rodents. Among NHPs, sleep/wake patterns have previously been studied in squirrel monkeys (Edgar et al., 1993; Klerman et al., 1999), rhesus macaques (Daley et al., 2006; Hsieh et al., 2008; Darbin et al., 2009; Tannenbaum et al., 2016), juvenile cynomologus macaques (Authier et al., 2014; Rachalski et al., 2014) and other species (Balzamo et al., 1977). The similar sleep/wake architecture of NHPs and humans has resulted in testing of preclinical compounds in NHPs for efficacy as well as safety prior to initiation of clinical studies (Uslaner et al., 2013; Tannenbaum et al., 2016).

The adenosine receptor antagonist caffeine (CAF) is undoubtedly the most widely-consumed, exogenous wake-promoting substance. Although CAF was initially thought to promote wakefulness by

Abbreviations: A1, Adenosine sub-type 1 receptor; A2a, Adenosine sub-type 2 receptor; CAF, Caffeine; EEG, Electroencephalogram; EMG, Electromyogram; i.m., Intramuscular; LMA, Locomotor activity; NHP, Non-human primate; NREM, Non-rapid eye movement; N1, NREM Stage 1; N2, NREM Stage 2; N3, NREM Stage 3; REM, Rapid eye movement; ROL, REM onset latency; SOL, Sleep onset latency; SEM, Standard error of mean; TST, Total sleep time; WASO, Wake after sleep onset

☆ The work was performed at SRI International. No clinical trials were performed.

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antagonizing the adenosine A1 receptor (Virus et al., 1990; Benington et al., 1995), more recent studies have implicated the adenosine A2a receptor (Huang et al., 2005). However, at doses generally consumed by humans, CAF produces its arousing effects by partial nonselective blockade of adenosine A1 and adenosine A2a receptors (Einöther and Giesbrecht, 2013; Clark and Landolt, 2017). Adenosine levels in the brain are positively correlated with the previous amount of waking (Porkka-Heiskanennen et al., 1997; 2003) and adenosine administration induces NREM sleep with high amplitude slow-waves (Schwierin et al., 1996; Benington et al., 1995). Hence, adenosine is one of the substances thought to be involved in homeostatic sleep regulation (Landolt, 2008). CAF is known to reduce or disturb sleep in a dose-dependent manner (Karacan et al., 1976; Hindmarch et al., 2000; Rosenthal et al., 1991; Yanik et al., 1987) and to reduce EEG slow wave activity in subsequent NREM sleep in humans and rodents (Carrier et al., 2009; Drapeau et al., 2006; Landolt et al., 1995a, 1995b, 2004; Schwierin et al., 1996). In squirrel monkeys, an adenosine receptor-mediated alerting effect of CAF is suggested by CAF attenuation of the dose-dependent decrease in lever-pressing under fixed interval schedules of food presentation induced by both selective as well as non-selective A1 and A2 adenosine receptor agonists (Howell and Byrd, 1993). In juvenile (2.5–3 y.o.) cynomologus macaques, CAF (10–30 mg/kg, p.o.) has been reported to decrease the duration of NREM and REM sleep, reduce low frequency EEG activity (1–8 Hz) during wakefulness, and to increase high frequency EEG activity (20–50 Hz) during NREM sleep (Authier et al., 2014).

We have recently identified EEG correlates for correct responses during a sustained attention task in adult cynomolgus macaques (Goonawardena et al., 2016). Although NMDA receptor antagonists were used in that particular study, we have also assessed the EEG in conjunction with the improved performance produced by CAF on sustained attention tasks, which has led us to examine the effects on CAF on sleep/wake and EEG characteristics in greater detail. In the present study, we report the sleep/wake architecture of middle-aged adult (9–11 y.o.) cynomolgus macaques across the night and assess the acute effects of CAF on nocturnal sleep. At the dose tested, CAF caused elevated wakefulness throughout the 12 h dark phase which led us to conduct a follow up 36 h recording after CAF administration. To our surprise, we found no evidence of sleep homeostasis despite a prolonged period of CAF-induced wakefulness during the 12 h dark phase.

2. Materials and methods

2.1. Animals

Male cynomolgus macaques (*Macaca fascicularis*; 9–11 years old; 7–10 kg) were maintained in constant environmental conditions (temperature 21 ± 3 °C; humidity 30–70%; 12:12 h light:dark cycle). All animals received a full daily regimen of standard certified commercial chow (Purina Animal Nutrition, Gray Summit, MO) supplemented with fresh fruit and vegetables and had access to water *ad libitum* in their home cage. Animals were individually housed but had visual, auditory and olfactory contact with other monkeys throughout the study, as well as access to chew toys. Videos and music were played in the housing room to provide environmental enrichment during the day. In accordance with Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and SRI guidelines, behavioral (e.g., activity level, locomotion, coordination) and clinical (e.g., appetite) signs were evaluated daily by a research scientist, particularly before and after surgery, and during the sleep studies. All efforts were made to minimize animal suffering and reduce the number of animals used. All procedures were reviewed and approved by the SRI International Animal Care and Use Committee (IACUC) and were in compliance with National Institutes of Health (NIH) guidelines for the care and use of laboratory animals. SRI International is an AAALAC-accredited institution.

2.2. Surgical procedures

Animals were fully anesthetized [telazole (4 mg/kg, i.m.) and 1% isoflurane] and placed on the surgical table in a dorsal recumbent position. Using aseptic surgical procedures, a longitudinal incision was made approximately 2 cm lateral to the *linea alba* by trained surgeons and a disk-shaped telemetry transmitter (D70-EEE; DSI, St-Paul, MN) was placed between the internal oblique muscle and the aponeurosis of the *transverse abdominis* muscle. The aponeurosis was sutured to hold the transmitter in place, the tips of EEG electrodes were individually sutured to prevent fluid from migrating up the leads, and the electrodes then were tunneled subcutaneously towards the back of the animal. The deeper layer of the abdominal incision was closed with a running subcutaneous pattern and, subsequently, the skin was closed with a running subcuticular pattern using absorbable sutures for both. Finally, several horizontal mattress stitches (3-0 non-absorbable Prolene; Ethicon US, LLC) were placed to remove tension from the incision.

Thereafter, the animal was placed into a stereotaxic frame and its head was secured with ear bars and a mouth/eye adaptor. The back and head/neck regions were aseptically prepared and a small skin incision was made on the back along the dorsal midline just below the scapular region. The fascia was blunt dissected down the animal's flank to retrieve the telemetry transmitter electrodes. A final skin incision was made on the head along the midline from the orbital ridge posteriorly to the occipital notch. Using a long hemostat, the biopotential leads were tunneled from the back incision to the head incision. The underlying connective tissue and muscles were incised to expose the skull, and retractors were used to hold the skin and muscle in place. Utilizing a stereotaxic approach, EEG electrodes were placed in the left and right frontal cortices according to the 10–20 system in humans and referenced to electrodes placed over the occipital cortex (left Fp1: AP +18.0 mm, ML +15.0 mm; left Oz reference: AP −22.0 mm, ML +3.0 mm from bregma; right Fp2: AP +18.0 mm, ML −15.0 mm; right Oz reference: AP −22.0 mm, ML: −3.0 mm from bregma). Coordinates were marked with a sterile pen and 4 burr holes were drilled through the skull in which stainless steel screws were inserted until the tips were estimated to touch the *dura mater*. The exposed wire tips were then wrapped around the 4 screws and silver epoxy was applied over the wire/screws to ensure good electrical conductivity. Once the epoxy was fully dried (approx. 15–20 min), all wires/screws were insulated with dental cement (FuliCEM resin modified glass ionomer cement; Net 32 Inc., Cary, NC). The EMG electrodes were implanted bilaterally into the superficial neck musculature (*trapezius*) and secured in place with non-absorbable (3-0) sutures. Once the dental cement was fully dried, the muscle, connective tissue, intra-dermal layer and skin were sutured separately (simple interrupted, 3-0 absorbable). All animals received perioperative anesthetic monitoring, postoperative analgesia for ≥ 72 h and antibiotics for 10–14 d, and were allowed a minimum of 21 d recovery period before experiments.

2.3. Electrophysiological and locomotor activity (LMA) recording

Telemetry receiver boards (RPC-1; DSI, St. Paul, MN) were mounted individually to each home cage to facilitate real-time EEG/EMG recording. Continuous acquisition of the EEG and EMG telemetry signals occurred at a sampling rate of 500 Hz using DataQuest A.R.T. (version 4.36; DSI); the frequency range of the D70-EEE telemetry transmitters was 0.3–100 Hz. LMA (counts per 0.25 min) was collected through the use of Actical accelerometers (Philips Respironics, NV) that were attached to each animal's collar. The Acticals have a processor with a 32 Hz sampling rate that enables recording of the frequency and intensity of the subject's movements in the vertical, horizontal and diagonal planes (counts/s). Following each experiment, Acticals were removed from the collars and the data were downloaded using the Actical software (version 3.1; Philips Respironics, Murrysville, PA) for offline analysis. The counts/s were then averaged across 15 s intervals