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Short communication

Paradoxical effects of the hypnotic Zolpidem in the neonatal ferret

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ABSTRACT

Hypnotic drugs designed to treat insomnia in adults are now increasingly used in children, but the effects of these compounds on neonatal sleep are poorly understood. We investigated the hypnotic effects of the commonly prescribed non-benzodiazepine sleep agent Zolpidem (*Ambien*™) on sleep architecture and electroencephalographic (EEG) activity in the neonatal ferret. Six ferret kits were surgically prepared for EEG/electromyographic (EMG) recordings using techniques adopted for use in neonatal animals. They were then administered in a counter-balanced design vehicle, or Zolpidem (2 mg/kg or 20 mg/kg) via intraperitoneal injection (1×/day over three days at 1 p.m.). Zolpidem did not increase non-rapid-eye-movement (NREM) or total sleep time. Instead Zolpidem reduced REM sleep and total sleep amounts and increased NREM sleep bout duration. Zolpidem also increased higher-frequency EEG energies during REM and NREM sleep and transiently produced a behavioral state that appeared intermediate between wake and sleep. Our findings demonstrate that hypnotics that improve sleep quality in adults may produce profoundly different behavioral changes in neonates.

Current estimates indicate that up to 40% of infants and 25–50% of pre-schoolers experience sleep disturbances and/or insomnia [26]. Although the US Food and Drug Administration has not approved the use of hypnotics in developing humans, physicians are often under intense pressure from parents to prescribe adult medications and parents themselves may 'medicate' their children without physician supervision [9,29]. Recent surveys suggest that in certain populations, as many as 25% of infants referred to a physician are given a hypnotic in the first year of life [9,27]. In one recent survey of 671 pediatricians, more than 75% had recommended nonprescription medications and 50% had prescribed a sleep medication to children with sleep problems (generally associated with acute pain, travel or special considerations such as autism) [27].

A wide variety of prescription and over the counter medications are reportedly being used to treat insomnia in children [9,27], but effective dosage schedules and safety profiles have not been determined for these drugs and they are often associated with adverse side effects. These include suppression of deep non-rapid-eye-movement (NREM) sleep and/or REM sleep, poor daytime cognitive function, rebound insomnia, tolerance and withdrawal symptoms and daytime sleepiness [9,14]. The suppression of the deeper stages of NREM sleep and REM sleep is particularly worrisome because both types of sleep may play important roles in brain maturation [6]. Moreover, it has recently been shown that even hypnotics with relatively benign effects on sleep architecture can profoundly impact developmental brain plasticity [30].

Despite these issues there is little basic pre-clinical research on this topic. For example, while the effects of antidepressants on neonatal sleep in rodents have been described [8,24] much less is known about how other medications impact neonatal sleep. The few studies that do exist suggest that some hypnotic agents may produce paradoxical effects in early life [23,33]. There is, however, only a single study of the hypnotic effects of commonly prescribed non-benzodiazepines such as Zolpidem (*Ambien*™) in developing animals [30]. In an earlier study, we showed that Zolpidem increases sleep continuity and produces EEG changes in weaning cats similar to effects reported in adult animals and humans [30]. The effects of Zolpidem at earlier ontogenetic stages are unknown.

We therefore characterized behavioral and electrophysiological changes following Zolpidem administration in the developing ferret (*Mustela putorius furo*). The ferret is particularly well-suited for developmental studies due to its extreme altriciality compared to other commonly used laboratory species. The ferret gestation period is relatively short (approximately 40 days, compared to 60–70 days for cats) [5]; thus ferrets exhibit fetal stages of development *ex utero* [15]. The relatively large size of the ferret kit also allows polysomnographic measurements during ontogenetic periods that are more difficult to perform in rodents or cats—which makes it increasingly a preferred animal model for studies of

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neurodevelopment. Sleep architecture, regulation and ontogenesis have also been recently characterized in the ferret and found to be similar to what is reported in these latter species [16,34].

The effects of Zolpidem were examined in three male and three female ferret kits obtained from four time-plugged Jills (Marshall Farms) at ages comparable to developmental stages associated with toddlers or pre-schoolers (based on weaning, presence of well-defined REM and NREM sleep [6,34] and maturation of GABAergic neurotransmission [4,11,12,32]). The Jills and weaned kits (weaning at approximately P33–35) were provided food and water ad lib and housed in our animal facility as described previously [34]. At postnatal (P) 30–31 ferret kits were surgically prepared for polysomnographic recordings and provided 4–5 days post-operative recovery with pain management and antibiotic treatments [34]. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

On P36, each ferret kit was weighed and individually connected to a light-weight, electrical cable attached to a commutator. They were then individually placed in custom-made incubators, provided KMR milk formula (and/or ferret chow) and were periodically groomed as described previously [34]. Following a 24-h habituation period (under the same housing conditions as in the home cage), continuous polysomnographic recordings were then made in each animal for the next three days (P37–P39). On the first day (P37), all kits were intraperitoneally (IP) injected with vehicle (DMSO 0.1 ml) at 1 p.m. Over the next two days, Zolpidem was administered in a counter-balanced design (2 mg/kg or 20 mg/kg in an equal volume of vehicle) at the same time of day (*i.e.* half the kits received 2 mg/kg at P38 and 20 mg/kg at P39; the other half had the reverse schedule). These doses were derived from those used previously in adult rodent studies [2,18,25]; the highest dose was selected based on pilot studies showing that intermediate doses (5 mg/kg, 10 mg/kg) had effects comparable to the low dose. All kits were weighed prior to each injection (mean ± SEM weights (grams) over the three days: 145.5 ± 0.12, 159 ± 0.13, 178 ± 0.13) and at the end of the last recording session in a series, the animals were deeply anesthetized with isoflorane in oxygen and euthanized with an IV overdose of Nembutal (150 mg/kg).

Polysomnographic recordings on each day (10 a.m.–7 p.m.) were amplified, filtered and digitized and recorded on a PC with commercial sleep acquisition/analysis software (Kissei Comtec America, Inc.), and scored in 8 s epochs as wakefulness, REM and NREM sleep as described previously [34]. After state assignments, we made the following measurements which were restricted to the 6 h following IP injections as performed in a study of Zolpidem in the adult mouse [18].

The mean percentage of each vigilance state was expressed as a % of total recording time (TRT) in 1 h bins immediately following DMSO or Zolpidem administration (*i.e.* from 1 p.m.–7 p.m.). The frequency and average durations of individual episodes of REM sleep, NREM sleep and wakefulness were also calculated in 1 h bins (minimum bout length was set at 8 s [34]).

Changes in sleep EEGs following Zolpidem administration were quantified using Fourier analyses [30,34]. For REM and NREM separately, mean spectra (0.5–40 Hz) were first divided by the corresponding mean spectra calculated from 10 a.m.–1 p.m. (before the IP injection) of the corresponding day to correct for day-to-day changes in the EEG due strictly to developmental trends. The resulting values following Zolpidem administration were then expressed as a % of the corrected time-matched DMSO values in 1 h bins. In order to asses EEG differences between a 'NREM-drowsy' state and wakefulness, REM and NREM sleep (in the last hour of the post-injection period, when drug effects had dissipated), a more fine-grained EEG analyses was used as described previously [16].

Data were first tested for normality using the Kolmogorov–Smirnov and equal variance tests (unless indicated otherwise, data are presented as means ± SEMs). Data sets that passed these tests were then assessed with Student's *t*-tests for planned, single comparisons. Mann–Whitney tests were used for non-parametric comparisons and a Holm-Sidek multiple comparison procedure was used in all other cases [13]. All statistics were calculated using SigmaStat software (Systat, Richmond, CA).

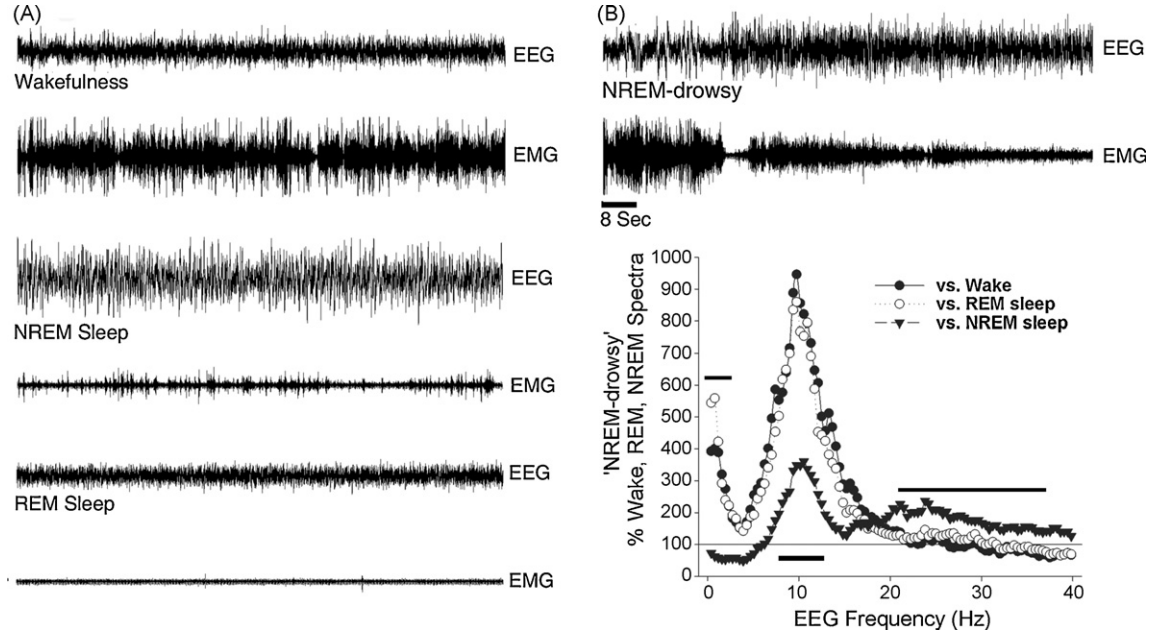

Fig. 1. Representative polygraphic recordings in the first hour following 20 mg/kg Zolpidem. (A) EEG and corresponding EMG traces from all vigilance states. (B) EEG and EMG traces during 'NREM-drowsy' state (upper panel). Lower panel shows EEG spectra in the NREM-drowsy state expressed as a % of either wakefulness, REM sleep or NREM sleep spectra. The black bars indicate EEG bands that were significantly different in NREM-drowsy relative to the other states (sigma activity, 10–15 Hz: Holm-Sidek, $p < 0.01$ vs. REM, NREM and wake; delta activity, 0.5–4.0 Hz: Holm-Sidek, $p < 0.01$, relative to wake or REM sleep; gamma activity, 20–40 Hz: Student's *t*-test, $p < 0.05$, relative to NREM sleep).