Short communication

Paradoxical effects of the hypnotic Zolpidem in the neonatal ferret

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A B S T R A C T

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.

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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 electrophysiologic 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...
neurodevelopment. Sleep architecture, regulation and ontogene-
sis 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,
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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 pro-
vided 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, pro-
vided 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. The resulting values following Zolpidem administra-
tion (i.e., 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 isoflurane 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 com-
mercial 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 follow-
ing 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 (min-
imum 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 cor-
responding 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 assess 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-Sidak multiple comparison procedure
was used in all other cases [13]. All statistics were calculated using
SigmaStat software (Systat, Richmond, CA).

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**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-Sidak, p < 0.01
vs. REM, NREM and wake; delta activity, 0.5–4.0 Hz: Holm-Sidak, 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).
Zolpidem produced a behavioral state that could not be clearly classified as wakefulness or sleep. This state was accompanied by relatively high to intermediate motor tone (e.g. the animals moved about the chamber for several minutes) combined with an EEG that appeared intermediate between NREM sleep and wakefulness (Fig. 1). Because of the mixture of relatively elevated motor tone (compared to sleep) and high amplitude, EEG waves, we refer to this state as ‘NREM-drowsy’ [30]. It was detectable immediately following Zolpidem treatment and less commonly observed at the lower dose (observed in two kits at 2 mg/kg; in all kits at 20 mg/kg). Spectral analyses showed that the EEG in this state was indeed intermediate between the three vigilance states (Fig. 1B).

Zolpidem did not produce adult-like changes in sleep architecture in neonatal ferrets. As shown in Table 1, both doses of Zolpidem reduced total sleep time. The highest dose also reduced REM sleep amounts and the frequency of waking, REM and NREM sleep bouts while increasing NREM bout duration (Table 2). The lower dose, however, had no effect on these parameters relative to vehicle. The main effect of Zolpidem on the sleep EEG was an increase in spectral energies in EEG bands >4.0 Hz (Fig. 2). In REM sleep, this was restricted to the first hour and to sigma (11–15 Hz) and theta (6–9 Hz) bands. In NREM sleep this was restricted to gamma bands (25–40 Hz), also in the first hour post-injection.

To summarize, Zolpidem produced changes in neonatal ferret sleep architecture and EEGs that were different than those typically reported in adult animals at comparable doses. In adult rodents [2,18,25], systemic administration of Zolpidem increases NREM sleep time, while reducing wake and REM sleep time [2,25]. Studies in adult mice and rats also show that Zolpidem generally leads to a large suppression of energy in faster EEG bands during NREM sleep [2,18,25]. The effects of Zolpidem on slower (delta) EEG energies are more complex. In mice, Zolpidem has been reported to reduce NREM delta [2], or have no effect [18]; in rats, Zolpidem leads to large increases in NREM delta [25]. In contrast, we find that Zolpidem does not significantly increase NREM sleep or total sleep time. We also find that Zolpidem increased energies in faster frequencies of the NREM sleep EEG, with no significant effects on NREM delta power. We also noted the appearance of a state that could not be clearly classified as wakefulness or sleep based on EMG/EEG measures (‘NREM-drowsy’), which may be related to automatic behavior (e.g. somnambulism) reported after Zolpidem administration [35].

The effects of Zolpidem in the neonatal ferret are consistent with reports that psychotropic medications designed for adults can produce paradoxical effects in early life [3,36]. For example, the effects of selective noradrenaline and serotonin uptake inhibitors on sleep/wake architecture change dramatically across the 2nd–3rd postnatal weeks in the rat [8]. Similar, age-dependent changes in sleep–wakefulness following serotonin depletion [1], or treatment with sleep-inducing peptides [10,28], pentobarbital [33] and glutamate receptor (NMDA) antagonists [21,22] are also reported in the literature.

The unpredictable nature of the neonatal response to neuroactive compounds results from several complex factors. These include the maturation of drug uptake mechanisms in the brain, liver metabolism, the binding affinities of neurotransmitter receptors

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**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>Wake (%)</th>
<th>NREM (%)</th>
<th>REM (%)</th>
<th>TST (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>22.2 ± 4.0</td>
<td>46.5 ± 2.7</td>
<td>30.0 ± 5.0</td>
<td>76.6 ± 3.9</td>
</tr>
<tr>
<td>Zolpidem 2 mg/kg</td>
<td>28.9 ± 7.1</td>
<td>40.3 ± 4.1</td>
<td>29.4 ± 5.3</td>
<td>69.8 ± 5.0*</td>
</tr>
<tr>
<td>Zolpidem 20 mg/kg</td>
<td>28.1 ± 8.5</td>
<td>55.5 ± 8.0(30.5 ± 6.5)#</td>
<td>11.6 ± 3.4*</td>
<td>66.6 ± 5.7*(42.1 ± 5.0)*#</td>
</tr>
</tbody>
</table>

Data (mean ± SEM) are expressed as a % of total recording time. Values in parentheses are state amounts with ‘NREM-drowsy’ considered separately (similar analyses at 2 mg/kg Zolpidem were not significant—data shown include NREM-drowsy). * indicates significant difference from vehicle (DMSO), # indicates significant difference between values with NREM-drowsy included vs. values without (Holm-Sidak, p < 0.05).

**Table 2**

<table>
<thead>
<tr>
<th></th>
<th>Wake (min)</th>
<th>Wake (freq)</th>
<th>NREM (min)</th>
<th>NREM (freq)</th>
<th>REM (min)</th>
<th>REM (freq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>2.3 ± 0.43</td>
<td>6.3 ± 0.74</td>
<td>3.86 ± 0.49</td>
<td>7.7 ± 0.8</td>
<td>4.3 ± 0.42</td>
<td>4.3 ± 0.6</td>
</tr>
<tr>
<td>Zolpidem 2 mg/kg</td>
<td>1.89 ± 0.45</td>
<td>7.8 ± 0.34</td>
<td>3.43 ± 0.50</td>
<td>7.7 ± 1.1</td>
<td>2.9 ± 0.48</td>
<td>6.2 ± 0.78</td>
</tr>
<tr>
<td>Zolpidem 20 mg/kg</td>
<td>3.7 ± 1.0</td>
<td>3.6 ± 0.36 **</td>
<td>7.65 ± 1.59**</td>
<td>2.7 ± 0.48**</td>
<td>3.25 ± 0.74</td>
<td>3.4 ± 0.36 #</td>
</tr>
</tbody>
</table>

Data are means (minutes: (min), frequency (freq) ± SEM. ** indicates significant difference between Zolpidem 20 mg/kg vs. vehicle (DMSO) and Zolpidem 2 mg/kg, # indicates significant difference between Zolpidem 20 mg/kg vs. 2 mg/kg (Holm-Sidak, p < 0.05).

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**Fig. 2.** The effects of Zolpidem on sleep EEG spectral energies. Data represent mean EEG spectral energies following 2 mg/kg (solid line) or 20 mg/kg Zolpidem (hashed line), expressed as a % of vehicle (VEH:DMSO) control injections in (A) REM sleep (lower line indicates significant difference between 2 mg/kg and 20 mg/kg vs. vehicle Holm-Sidak, p < 0.05) and (B) NREM sleep (lower line indicates significant difference between 20 mg/kg vs. vehicle Holm-Sidak, p < 0.05).
(as well as the particular 2nd-messenger, intracellular cascades triggered by their activation), and the maturation of arousal and sleep circuitry: all of which undergo major changes during perinatal development [6,7,19,31]. Consequently, the precise mechanisms mediating the paradoxical effects of Zolpidem in the neonate are presently unknown. However, they likely include differences in the metabolism of Zolpidem because in comparison to adult animals, the effects of Zolpidem on neonatal sleep structure dissipated relatively rapidly [25]. They also may reflect developmental changes in the composition of the GABA receptor [17,20]. In rodents, these events take place during the 2nd–3rd postnatal week, which based on comparative developmental markers (e.g. eye-opening, maturation of REM and NREM sleep), is comparable to the 4th–5th postnatal week in the ferret [15,34]. Therefore, prior to a certain developmental age, modulation of the GABA receptor via α1 binding may produce a mixture of soporific and arousing effects in neonates.

Acknowledgement

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References

[34] Thurber A, Jha SK, Coleman T, Frank MG. A preliminary study of sleep ontoge.