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Preclinical Evaluation of Behavioral Deficits Following Hypnotic Drug Treatment

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HALPERIN, J M , J CANON AND L C IORIO *Preclinical evaluation of behavioral deficits following hypnotic drug treatment* PHARMACOL BIOCHEM BEHAV 24(4) 875–878, 1986 —Most hypnotic medications result in performance decrements, or "hangover effects," the morning after bedtime ingestion However, the severity of hangover effects with new compounds is difficult to predict prior to clinical trials This paper describes a preclinical procedure that is predictive of human hangover effects following bedtime ingestion of hypnotic medication Four cebus monkeys were shaped to discriminate between two levers such that rapid responses following the cue light maximized the probability of reinforcement Subsequently, performance was evaluated one hr (immediate condition) and 8¾ hr (delayed condition) after the administration of flurazepam, triazolam, pentobarbital and placebo All drugs caused dose-dependent performance decrements in both conditions. Significantly higher doses of triazolam were required to impair performance during the delayed condition as compared to the immediate condition, but no such differences occurred with either flurazepam or pentobarbital These findings parallel human data which indicate that sleep-inducing doses of flurazepam and many barbiturates, but not triazolam, cause performance decrements the morning after bedtime ingestion

Hypnotics Sleep Flurazepam Triazolam Pentobarbital Monkeys Hangover Performance

THE preclinical evaluation of drugs for hypnotic potential has been carried out successfully using a variety of different techniques ranging from simple behavioral assessments in rodents [1, 3, 8] to more sophisticated methods utilizing polygraph recordings and animal models of insomina [4,5]. However, sleep-inducing efficacy is only one of several necessary requirements for a useful hypnotic drug Recently, much attention has focused on human performance decrements, or "hangover effects," following bedtime administration of these medications This paper describes a preclinical procedure designed to predict the magnitude of human performance decrements on the day following night-time ingestion of sleep-inducing drugs

After an extensive review of the literature, Johnson and Chernick [6] concluded that at some doses all hypnotic medications produce performance decrements following night-time ingestion, and that although long-acting drugs generally caused greater decrements in performance, half-life data were not consistent Roth, Krammer and Lutz [7] examined human performance on several psychomotor and cognitive tasks following the administration of clinical doses of three hypnotic drugs, flurazepam, triazolam and secobarbital They found that all three drugs resulted in significant impairment relative to placebo when the subjects were awakened in the middle of the night (3 5 hours after drug ingestion). However, in the morning (10 hours post-ingestion) only those who received 30 mg of flurazepam were significantly impaired as compared to placebo.

It would be extremely advantageous to be able to predict the extent of performance decrements following bedtime ingestion of hypnotic medications prior to clinical trials, in order to develop a drug that would be both effective and hangover-free However, the only reference to such a task is that of Gogerty [2], who reported that cebus monkey performance on a DRL schedule was disrupted nine hours after sleep-inducing doses of flurazepam, but not methaqualone

Based on polygraphic data reported by Gogerty [2], the cebus monkey *(Cebus appella)* is the species of choice for developing a preclinical screening procedure for evaluating hypnotic medications Not only do they have a diurnal sleep cycle which is similar to humans, but the data indicate that they respond to hypnotic medication in a manner similar to man with respect to both potency and duration of action.

This paper describes an operant procedure, using cebus monkeys, that yields data which is highly predictive of hypnotic hangover effects in man. The task evaluates both decision-making and reaction time in the subjects. Three different hypnotics were tested one hour after drug administration, to assess immediate drug effects, and 8¾ hours post-administration, to evaluate delayed effects

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TABLE 1

PERCENTAGE OF CORRECT RESPONSES AT EACH TIME DURATION FOR 4 MONKEYS DURING VEHICLE DAYS

Time Duration*	Mean % Correct	SD
0 25	0 92	2 3
0 50	44 49	24 6
1 0	81 01	14 4
2 0	89 41	10 5
4 0	91 62	10 6

*In seconds

TABLE 2

MINIMAL EFFECTIVE DOSE (mg/kg, PO) FOR CEBUS MONKEYS TREATED WITH HYPNOTICS UNDER IMMEDIATE (I) TESTING AND DELAYED (D) CONDITIONS

	Flurazepam		Triazolam*		Sodium Pentobarbital	
Cebus No	I	D	I	D	I	D
27	2 5	2 5	0 0125	0 05	5 0	10 0
37	2 5	2 5	0 025	0 05	7 5	5 0
47	0 625	2 5	0 0125	0 1	5 0	20 0†
197	2 5	1 25	0 0125	0 1	5 0	12 5
Mean	2 03	2 19	0 016	0 075	5 63	11 88

*MED for I and D conditions differ significantly, paired t-test, $t(3)=10\ 27$, $p<0\ 01$

†This number is likely to be spuriously high since 10 mg/kg was inadvertantly never tested in this condition

TABLE 3

RL50'S* PRODUCED BY THE MED FOR 3 HYPNOTICS DURING IMMEDIATE (I) AND DELAYED (D) TESTING IN 4 MONKEYS

	Vehicle		Flura-zepam		Tria-zolam		Sodium Pento-barbital	
Cebus No	Mean	95% conf limits	I	D	I	D	I	D
27	0 69	0 66–0 72	1 12	0 80	0 73	0 91	0 81	0 77
37	0 54	0 48–0 61	0 90	2 00	0 92	0 82	0 90	0 74
47	0 52	0 47–0 57	0 64	0 66	0 60	0 80	0 60	1 46
197	0 60	0 46–0 74	3 00	0 94	0 86	0 82	0 78	0 79

*In seconds

TABLE 4

IMPAIRMENT RATIO (IR)* FOR CEBUS MONKEYS TREATED WITH FLURAZEPAM, TRIAZOLAM AND SODIUM PENTOBARBITAL†

Cebus No	Flura-zepam	Tria-zolam	Sodium pentobar-bital
27	1 0	4 0	2 0
37	1 0	2 0	0 67
47	4 0	8 0	4 0‡
197	0 5	8 0	2 5
Mean	1 63	5 50	2 29

*IR=MED Delayed/MED Immediate

†$F(2,6)=8\ 49$, $p<0\ 05$

‡May be spuriously high, see footnote in Table 2

METHOD

Subjects

The subjects were four adult, male, cebus monkeys *(Cebus appella)* ranging in weight from 2.6 to 3 9 kg Throughout the experiment each monkey was housed individually under a 16-hr light, 8-hr dark, light-dark cycle Purina Monkey Chow was available ad lib However, access to water was restricted to 1/2 hour per day.

Procedure

The monkeys were shaped to discriminate between two adjacent levers mounted on the wall of their chamber. Depression of the lever over which a cue light was illuminated was reinforced with 0 5 ml unsweetened orange juice that was delivered by a liquid dipper mounted on the same wall of the chamber as the levers. Responses following the offset of the cue light, which remained illuminated for either 0.25, 0 5, 1 0, 2 0 or 4.0 sec, were not reinforced.

Within each test session, which lasted approximately 90 min, the localization of the cue light and the duration of illumination were randomly distributed over 250 trials such that there were 50 trials per time-duration, with 25 of them correct on each lever. Each trial was preceded by a 0.5 sec warning tone 5 sec prior to the onset of the cue light A trial was terminated at the offset of the cue light or following the monkeys' first response, whichever came first. There was a 20-sec intertrial interval.

The percent of correct responses was determined for each cue light illumination time-duration The reaction latency (RL50), defined as that duration at which each monkey responded correctly 50% of the time, was calculated using linear interpolation. Trials that were not responded to correctly were divided into discrimination and omission errors. Omission errors resulted from either a lack of response or a response after the offset of the cue light Discrimination errors occurred when the incorrect lever was depressed while the cue light was still illuminated

Two separate paradigms using this operant procedure were utilized to test immediate and delayed drug effects To test immediate drug effects, monkeys received either drug or vehicle one hour prior to the test session, which was conducted during the lights-on part of the light-dark cycle

To test delayed effects, drug or vehicle was administered 30 min prior to the onset of the dark period The operant test session began 8 3/4 hours later, which was 15 min after the onset of the light period Although EEG was not recorded during the dark period, both behavioral observations and EEG data from other laboratories [2] suggest that the dark period is the normal sleeping time for cebus monkeys Thus, this paradigm was designed to be analogous to hypnotic intake 30 min prior to bedtime, and behavioral testing 15 min after awakening the following morning

Three hypnotics were evaluated using both of these paradigms, flurazepam, sodium pentobarbital and triazolam All drugs were suspended in a 0.4% methylcellulose solution and administered orally at a volume of 2 ml/kg The dose range varied somewhat for each animal and condition depending upon individual differences in sensitivity. However, each monkey received between 3 and 5 logarithmically spaced doses of each drug The overall range of doses for each drug were flurazepam, 0 625–20 mg/kg, sodium pentobarbital, 5–40 mg/kg, and triazolam, 0 0125–0 2 mg/kg

Monkeys were tested daily in the operant paradigm. Each drug day was preceded by a vehicle day. Following drug days the monkeys received neither drug nor vehicle until their level of responding returned to pre-drug levels

The RL50 was determined for all test sessions that were preceded by either vehicle or drug injection. The minimal effective dose (MED), defined as the lowest dose of each drug that increased the RL50 beyond the 95% confidence limits of the vehicle days, was determined separately for each monkey during immediate and delayed conditions. An impairment ratio (IR), defined as the MED for the delayed test divided by the MED for the immediate test (IR=MED Delayed/MED Immediate) was determined for each drug in each monkey. The IR for the three hypnotics were then compared using a repeated measures one-way analysis of variance

RESULTS

Vehicle treated monkeys responded correctly more than 90% of the time at the longest cue light duration (4 sec) and almost never at the shortest duration (0.25 sec), with the percentage of correct responses varying as a function of the duration of the light (see Table 1). Those trials that were not responded to correctly were comprised almost entirely of omission errors. Less than 5% of the non-reinforced trials contained discrimination errors. There was no significant difference ($p>0.10$) on vehicle days between the RL50's for the immediate and delayed paradigms The mean (S.E) RL50's were 0 58 (0 13) sec and 0.60 (0 09) sec, respectively.

All three drugs caused dose-dependent increases in the RL50 during both the immediate and delayed conditions. However, as shown in Table 2, only triazolam was significantly ($p<0$ 05) more potent one hour after drug administration as compared to 8 3/4 hours later There were no significant differences ($p>0$ 10) between the. minimal effective doses of flurazepam or sodium pentobarbital required to cause behavioral disruption in the immediate and delayed paradigms Table 3 shows the RL50's produced by the MED of each drug along with the RL50 and 95% confidence limits which were derived from vehicle data.

As shown in Table 4, there was a significant difference in IR between the drugs, $F(2,6)=8$ 49, $p<0$ 05. Post hoc analyses indicated that the IR was significantly ($p<0.05$) higher for triazolam as compared to either flurazepam or sodium pentobarbital The dose of triazolam required to significantly increase the RL50 on the delayed test was more than five times that required to raise the RL50 upon immediate testing (IR=5 5), whereas only minimal increases in the doses of flurazepam and sodium pentobarbital above the MED for the immediate test resulted in performance decrements 8 3/4 hours later

DISCUSSION

The data indicate that this operant procedure in cebus monkeys may be useful for predicting behavioral deficits associated with hypnotic drugs prior to clinical trials. It yields results that are both dose-dependent and highly predictive of human potency

As in man, performance decrements occur during the immediate testing condition at lowest doses for triazolam, with the barbiturate requiring the highest dose. Also, as in humans, the data indicate the greatest severity of delayed impairment with flurazepam, with minimal effects following triazolam Of the three compounds tested, only triazolam

required significantly higher doses to cause performance decrements 8¾ hr post-drug as compared to 1 hr post-drug This suggests, as one would expect based upon the pharmacokinetics of these compounds, that flurazepam and pentobarbital have a longer duration of action with regard to behavioral disruption than triazolam, and are therefore more likely to result in performance decrement or hangover on the day after bedtime drug administration

The impairment ratio for triazolam was significantly higher than that of either flurazepam or pentobarbital Whereas doses greater than five times the minimal effective dose of triazolam were required to cause delayed deficits, doubling the dose of pentobarbital, and only a two thirds increase in the dose of flurazepam resulted in significant performance decrements during delayed testing

We have described and evaluated an operant procedure that can be useful for evaluating behavioral side effects following the use of hypnotic drugs Further evaluation of this technique may indicate that it can also be useful for predicting behavioral side-effect potential of other, non-hypnotic drugs

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