Psychopharmacologia (Berl.) 40, 313–318 (1975)

The Effects of Age and Illumination on the Dose-Response Curves for Three Stimulants*

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Received April 5, 1974

Abstract. The dose-response relationships for three stimulants have been explored. These drugs have been shown to differ in potency and, with successive doubling of doses, have been found to have dose-response curves of differing slopes. In addition, the relationship between dose and activity level was not the same for younger and older rats. The relationship between dosage of both *d*-amphetamine and methylphenidate and locomotor activity was not the same in the light and the dark. The latter finding suggests a difference between these two stimulants and the third stimulant studied, caffeine, whose effects where unaltered by ambient illumination level.

Key words: *d*-Amphetamine — Methylphenidate — Caffeine — Age — Illumination — Activity.

Dextroamphetamine and methylphenidate are central nervous system stimulants closely related in structure and clinical use (Stein and Weiss, 1970). Clinically the two drugs are used interchangeably in the treatment of hyperactivity, or hyperkinesis, in children (Fischer and Wilson, 1971; Knights and Hinton, 1969; Werry and Sprague, 1970). Cole (1969) has suggested that *d*-amphetamine may have its subduing effect on hyperkinetic children by increasing their attention rather than directly sedating the child, while Fischer and Wilson (1971) have offered a similar hypothesis for the action of methylphenidate. Previous work (Isaac, 1971; Seegal and Isaac, 1971) has shown that the stimulant effects of *d*-amphetamine on the activity of the rat are most pronounced in the light. If the increased attention noted with *d*-amphetamine and methylphenidate is a function of an alteration in the effects of sensory input to the organism, the effects of methylphenidate also would not be expected to be independent of illumination.

Although caffeine is also classed as a stimulant (Stein and Weiss, 1970), Stinnette and Isaac (1973) found that caffeine and *d*-amphetamine interact differently with illumination in the Squirrel monkey. The present study extends these findings to a nocturnal animal.

* This work was supported by research grant (MH 22140) from the National Institute of Mental Health.

To study the illumination—drug relationship among three different stimulants under comparable conditions, dose-response curves were obtained for each of the drugs. Subjects of two age levels were studied to increase the generality of the findings.

Methods

Subjects. Sixty Cherokee-SD, male, albino rats were used as subjects. Half of the animals were 30 days old at the beginning of adaptation and the other half were 150 days old. The behavioral arousal of the younger animals would be expected to approach the adult level since the inhibitory systems controlling arousal are judged to have matured by the age of 25 days (Mabry and Campbell, 1974); the older animals are just beyond the period of rapid growth. All animals were individually housed in the colony room on a 12 hrs light-12 hrs dark illumination schedule between experimental sessions. Food and water ware available *ad libitum* in the colony room.

Apparatus. Activity was measured by an infrared photocell device bisecting a plastic mouse breeding cage 45 × 24 × 15 cm high, covered with hardware cloth. A response was recorded by electromechanical counters each time the rat's body interrupted the light beam. The activity chambers were placed in individual sound attenuated cubicles in a sound attenuated room. During the light condition each cubicle was illuminated by a 20 watt, cool white, fluorescent bulb mounted 29 cm above the center of the floor of the activity cage. Each cubicle was thus illuminated by approximately 180 foot candles of light at the floor of the activity cage. The room was totally dark during the dark condition. The temperature in the colony room and experimental chamber was maintained between 22 and 24 degrees centigrade throughout the experiment.

Procedure. All animals were placed in the activity chamber for 65 min per day. The first five minutes served as an adaptation period and data were recorded for the last 60 min of the session only. All animals were run at the same time every day in the same experimental cubicle. Light and dark conditions were alternated daily throughout the experiment.

All animals were adapted to the procedure and apparatus for 10 days. On days one through four each animal was placed in the experimental chamber for 65 min and then returned to his home cage. On days five through ten all animals were injected with sterile water at a volume of 1 ml/kg of body weight and placed in the activity chamber for 65 min. All injections were given 5—10 min before the subjects were placed in the experimental chambers. Throughout the study the thirty-day-old animals were weighed every third day because of their fast growth rate while the 150-day-old rats were weighed every sixth day. New dose levels were computed each time the animals were weighed.

The animals were randomly assigned to one of three drug[1] conditions for the remainder of the study: *d*-amphetamine sulfate, methylphenidate hydrochloride, or caffeine sodium benzoate, and on the 11th day testing began. Animals were tested in groups of ten so that each group had three animals for each of two drugs and four animals for the third drug. Drug doses were randomly assigned to days so that

[1] The drugs used in this study were generously provided by the following companies: *d*-amphetamine sulfate by Smith, Kline and French Laboratories, methylphenidate hydrochloride by Ciba-Geigy Corporation, and caffeine sodium benzoate by Eli Lily & Company.

all animals received the same dose level on a given day. A different sequence of doses was used for each replication. Five levels of each drug plus a placebo were used. *d*-Amphetamine doses were 0, 0.2, 0.4, 0.8, 1.6 and 3.2 mg/kg. Methylphenidate doses were double those for *d*-amphetamine or 0, 0.4, 0.8, 1.6, 3.2 and 6.4 mg/kg. Caffeine doses were ten times those of *d*-amphetamine or 0, 2.0, 4.0, 8.0, 16.0, and 32.0 mg/kg. The lowest doses of the drugs are within the range of lower clinical doses commonly used with hyperkinetic children (Werry and Sprague, 1970; Ritchie, 1965). Drugs were mixed with sterile water to provide an equal volume intraperitoneal injection of 1 ml/kg of body weight for all levels of the drugs. A replication consisted of all doses in both the light and dark conditions for a total of 12 days per replication. Three complete replications were done for a total of 36 consecutive days.

Results

The raw scores were transformed by the $\sqrt{X + 1}$ transformation, as suggested by Edwards (1961) for frequency data, and evaluated by analysis of variance. In all cases, those interactions including the subject variable were combined with the residual to form a pooled estimate of the population variance (Edwards, 1961).

Analysis of the data obtained from the last six days of the adaptation period demonstrated that the three groups to be administered different drugs did not differ in their pre-drug activity levels. The younger animals were more active than the older ($F = 16.31$; $df = 1, 54$; $P < 0.01$),with both groups more active in the dark than in the light ($F = 58.37$; $df = 1, 294$; $P < 0.01$). The younger subjects showed a greater illumination effect than the older, resulting in a significant interaction ($F = 14.31$; $df = 1, 294$; $P < 0.01$).

An analysis of variance performed on the data obtained with all three drugs was difficult to interpret because of multiple higher-order interactions involving the effects of the different drugs. The effects of the drugs were not equivalent ($F = 27.62$; $df = 10, 1890$; $P < 0.01$). For this reason, separate analyses of variance were performed upon the data obtained with each drug.

Activity levels increased over the three replications for the three drug groups. Since this was true of their performance on non-drug days as well as on those days when drugs were administered, it would suggest a continuing adaptation of the subjects to the experimental conditions rather than a cumulative effect of the drug injections. In no case was the dose-replication interaction statistically significant.

The simple effect of age was not found to be a significant variable in the analyses of the three drug treatment groups. Activity was significantly influenced by amphetamine ($F = 110.52$; $df = 5, 630$; $P < 0.01$), methylphenidate ($F = 148.40$; $df = 5, 630$; $P < 0.01$), and caffeine ($F = 79.93$; $df = 5, 630$; $P < 0.01$). There was a statistically significant interaction between amphetamine doses and age ($F = 3.52$; $df = 5, 630$;

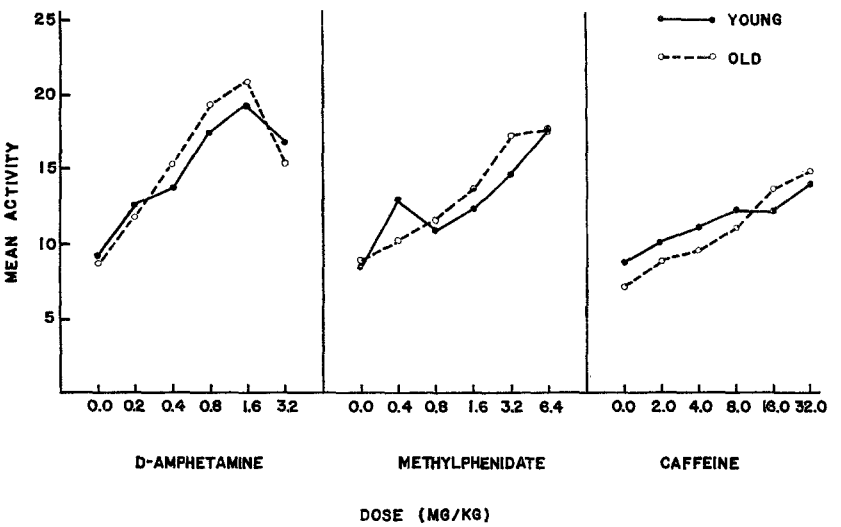

Fig. 1. Activity levels as a function of dose levels of *d*-amphetamine, methylphenidate, caffeine and age

$P < 0.01$), and caffeine doses and age ($F = 6.76$; $df = 5, 630$; $P < 0.01$) (Fig. 1).

The simple effect of illumination was not significant in combination with amphetamine, but the dose and illumination interaction was significant ($F = 3{,}50$; $df = 5, 630$; $P < 0.01$). With methylphenidate the illumination effect was significant ($F = 4.53$; $df = 1, 630$; $P < 0.05$), as was the interaction between dose and illumination ($F = 2.43$; $df = 5, 630$; $P < 0.05$). The simple effect of illumination in combination with caffeine was significant ($F = 66.37$; $df = 1, 630$; $P < 0.01$), while the interaction between dose and illumination did not reach significance. Except with amphetamine, the total activity was greater in the dark than in the light. This light-dark difference was seen in all three groups on the days that placebo injections were given. The interaction seen between dose and illumination with amphetamine and methylphenidate indicated a steeper dose-effect curve in the light than in the dark (Fig. 2).

Discussion

It is apparent that the three stimulants studied, *d*-amphetamine sulfate, methylphenidate hydrochloride, and caffeine sodium benzoate, are not equally potent in increasing the locomotor activity in the rat. Further, increasing the doses in a doubling fashion did not produce parallel increases in the behavior measured. Thus, a simple ratio cannot be used to compare their effectiveness in altering activity level.

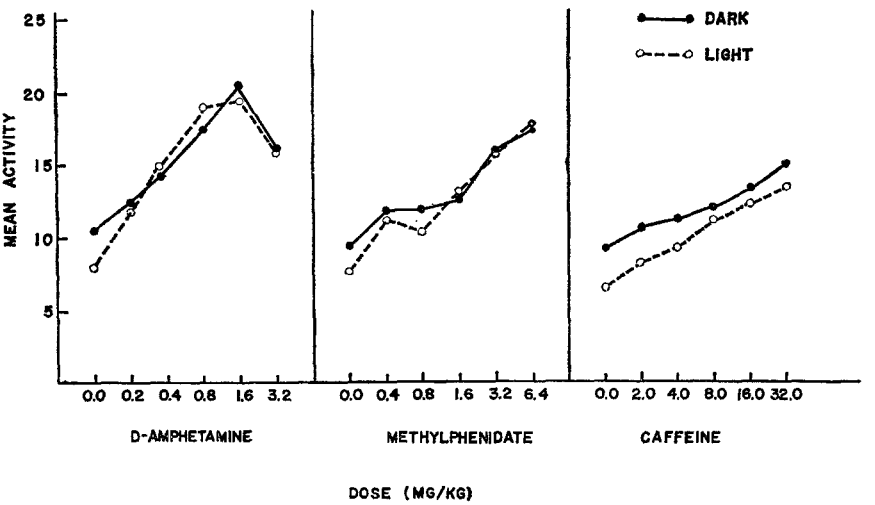

Fig. 2. Activity levels as a function of dose levels of *d*-amphetamine, methylphenidate, caffeine and illumination

In addition, the age of the subjects must be considered in any statement concerning dose-response relationships for each of the drugs, or in any comparisons among the drugs. The younger subjects were more sensitive to the lower doses of all three drugs and showed a lesser sensitivity to the higher levels.

The observed behavioral toxicity found with the highest dose of *d*-amphetamine is consistent with the findings of Adler (1961) whose use of the less potent racemic form of the drug may account for the decrease in activity at higher doses than those of the present study. The unique response of the younger subjects to the smallest dose of methylphenidate (0.4 mg/kg) produced an increase in activity equivalent to the increase produced by the smallest dose of *d*-amphetamine (0.2 mg/kg).

The effects of two of the drugs studied, *d*-amphetamine and methylphenyldate, were not independent of the ambient illumination condition. Both drugs produced greater changes in activity under the light condition and had a less pronounced effect in the dark. The non-independence of *d*-amphetamine and illumination resulted in an elimination of the light-dark effect when this drug was administered. The independence of caffeine effects and ambient illumination conditions shows that this is not a general effect of stimulants. It has been suggested (Alexander and Isaac, 1965) that *d*-amphetamine exerts its effect upon locomotor activity by reducing the effectiveness of illumination upon the arousal mechanism. This hypothesis received support in the comparison of the

drug as it influenced the activity of diurnal and nocturnal monkeys in opposite directions in the presence of light (Isaac and Troelstrup, 1969). That is, in both groups of monkeys the effect of *d*-amphetamine upon activity was the same as reducing illumination levels. The finding that the effects of methylphenidate, but not caffeine, are also influenced by ambient illumination conditions suggests different mechanisms by which stimulants exert their effects.

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