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BRIEF COMMUNICATION

Circadian Variation in an Amphetamine Induced Motor Response

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URBA–HOLMGREN, R., B. HOLMGREN AND M. AGUIAR. *Circadian variation in an amphetamine-induced motor response.* PHARMAC. BIOCHEM. BEHAV. 7(6) 571–572, 1977. – Significant diurnal variation in occurrence and duration of D-amphetamine induced head-shaking were observed in 9-day-old rats. The lowest values were obtained near noon, and the highest around midnight. These variations should be considered if head-shaking is to be used as a quantitative neuropharmacological test.

Circadian rhythm Head-shaking D-amphetamine

IT IS WELL known that diurnal cycles exist in the effectiveness of several drugs, due either to cyclic changes in drug metabolism, distribution and excretion, or to cyclic variations in the sensitivity of the target tissues to the particular drugs [6].

While studying D-amphetamine induced head-shaking in infant rats [3] we observed a tendency towards a decrease in the occurrence and duration of this motor pattern when the experiments were performed near noon or in early afternoon hours. Other investigators have reported circadian rhythms for the content of noradrenaline in the brain [2] and for different behaviors in which monoaminergic pathways are involved [5, 8, 9]. We considered that it was important to establish if diurnal changes also existed in head-shaking induced by amphetamine in infant rats, in order to develop a reliable neuropharmacological test based on this motor item.

METHOD

The experiments were performed in rats of a Wistar strain, having reached the age of 9 days (± 4 hr) at the beginning of the test. No special measures were taken in relation to dated pregnancy nor induction of labor at particular hours. Every litter was reduced to eight rats 24 hr after birth and was subjected, in the laboratory, to a light-darkness regime of 9/15 hr: The artificial light period extended from 0800 to 1700 hr, the dark from 1700 to 0800. Before arrival to the laboratory, the pregnant mothers were maintained in the Animal House on a natural light-dark schedule of approximately 12/12 hr. Animals from each litter were distributed for head-shaking tests at consecutive three hr intervals. During each hour 15 to 20 animals were tested, the average results in each time point corresponding to animals from 7 to 8 different litters. Thus, our results correspond to the observation of a population of 9-day-old rats in which the individuals are tested at different hours throughout the day.

Head-shaking was induced with D-amphetamine sulphate (5 mg/kg, the dose referred to the salt) injected intraperitoneally in saline solution and in a total volume of 0.01 ml/g bodyweight. The occurrence and total duration of the the headshaking episodes were quantified, during a one hr observation period [3].

RESULTS AND DISCUSSION

The results, illustrated in Fig. 1, show strong time dependence of both occurrence and total duration of head-shaking. Occurrence values in tests between 1100 and 1500 hr were significantly different from those obtained in tests begun at 0800 or 0900 as follows: 1100 hr, $p<0.02$; 1200 and 1300 hr, $p<0.001$; 1400 hr, $p<0.005$ and 1500 hr, $p<0.02$ (Chi^2 tests). Occurrence of head-shaking at the other hours studied did not differ from those at 0800–0900 hr. On the other hand, when total duration of head-shaking time is analyzed with a Kruskal-Wallis test [7] the results point to heterogeneity in the population ($p<0.001$). Since inspection of the data suggested that they might be fitted by a periodic function, a harmonic regression analysis was carried out [1]. The analysis of variance F-test ($F = 0.4$, with $n_1 = 17$ and $n_2 = 19$) indicates that an adequate fit to the experimental material could be obtained by the function: $y = A_0 \sin(2\pi \omega t + \rho) + B$ with a period of 24 hr (A, amplitude; ω period; t, time of the day; ρ, phase angle and B, minimum value of the curve). The close agreement between predicted and observed values can be seen in the figure.

The chronological coincidence of high head-shaking values between midnight and 0400 hr, with the rise in

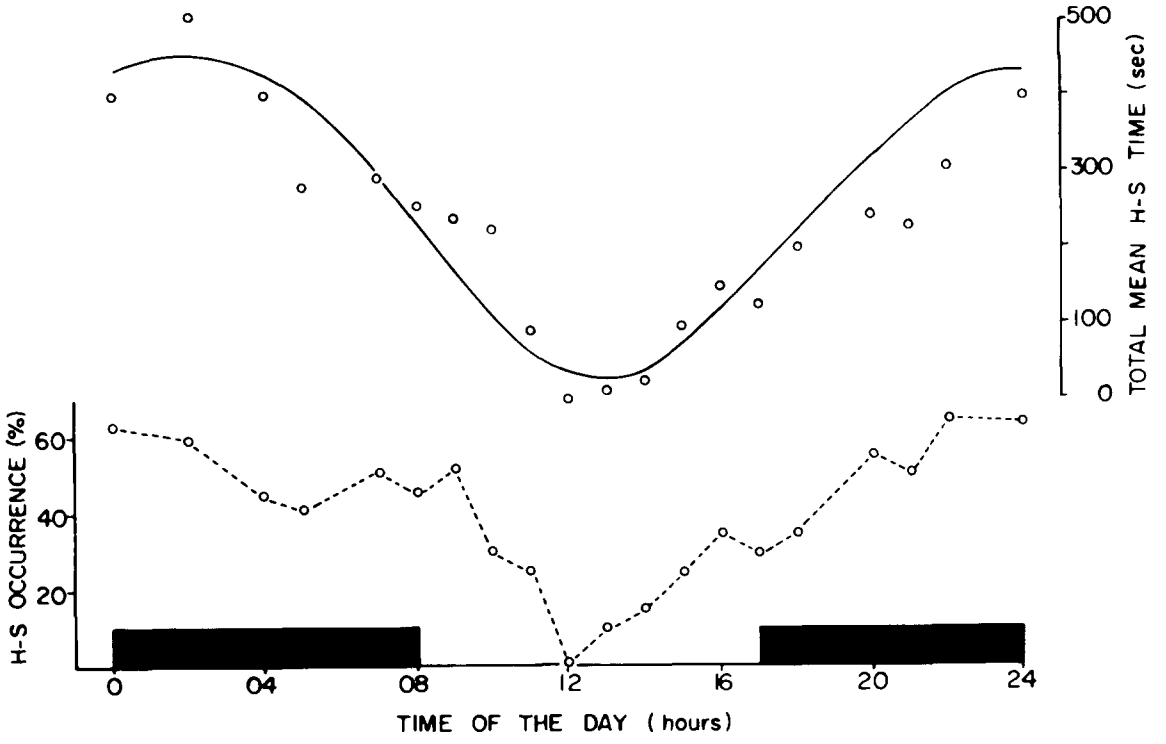

FIG. 1. Circadian variations of amphetamine induced head-shaking. Ordinates: Lower curve, H–S occurrence (%); Upper curve, Total Mean H–S Time (seconds). The line drawn corresponds to the periodic function described in the text. o = experimental data.

noradrenaline in the mid-brain, described by Friedmann and Walker [2] in normal adult rats, is suggestive of the importance which has been assigned to noradrenergic mechanisms in head-shaking [4]. Whatever might be the causes of significant diurnal variations in this simple motor response to D-amphetamine, these factors impose due consideration be given to the time of the day in which the experiments are performed, in order to reduce variance or for valid comparison of the results to be possible. As an example, previously reported results [3,4] which included data from tests performed from 0800 to 1200 hr might have led to higher average values and lower variance if the tests performed after 1000 hr had been excluded.

Therefore, if amphetamine induced head-shaking is to be used as a neuropharmacological assay, it seems advisable to perform the experiments within restricted hourly margins, and at times in the day in which the behavior is at intermediate levels. This would enable one to observe either facilitatory or inhibitory effects, or both.

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