ony, whereas the majority of the Ptv group are among the last 66 chimpanzees added to the colony (Table 1). Furthermore, the frequencies of the variant transferrin types differ markedly when the older half of the colony is compared to the newer type. The tabulation of phenotypes in Table 1 reveals that in the older half of the colony the proportions of the different transferrins are 59C:27D:15A:-2B:1E, whereas in the newer half the proportions are 109C:8B:1A. Thus the transferrin polymorphism is not nearly as marked in the half of the colony containing the majority of Ptv chimpanzees (the newer half) as in the half containing the majority of the Pts animals (the older half).

The transferrin polymorphism in the Ptt group is stronger than in the Ptv group, but not as strong as in the Pts group. The three most common transferrin alleles in the colony have frequencies in the Ptt group which are close to the average of their frequencies in the Ptv and Pts groups. The pattern of intermediate gene frequencies in the Ptt group (Table 2) correlates with the intermediate geographic location ascribed by Hill (*1*) to *P. t. satyrus* (*P. t. troglodytes*), which lies between *P. t. verus* at the far western side of the chimpanzees' range and *P. t. schweinfurthi* at the center and eastern side of the range. This correlation suggests that the morphological traits used by Hill to classify the Holloman chimpanzees into racial types are good indicators of the different conspecific chimpanzee populations as they occur in their natural habitats.

Syner (*12*) discovered a variant of the lactate dehydrogenase B subunit and of glucose-6-phosphate dehydrogenase in two different individuals of the Pts group; Hoffman (*13*) found a variant of the hemoglobin alpha chain in a third Pts chimpanzee and a variant of the hemoglobin beta chain in another Pts chimpanzee. In addition to these rare variant alleles found only in the Pts group of the Holloman colony, polymorphisms of the red-cell enzymes, phosphoglucomutase and adenylatekinase, were found by Tashian (*14*) throughout the colony, with the frequency of the polymorphisms being greater in the Pts group than in the Ptv group. These results suggest that genetic variability is extremely high in the chimpanzee population from particular geographic areas (presumably in the eastern or central part of the range) and relatively low in the population from certain other areas (presumably at the western periphery of the species distribution). A survey of the incidence of polymorphic protein macromolecules in the native chimpanzee populations from localities of differing ecological and geographic characteristics would clarify the genetic and natural selective mechanisms operative in chimpanzee evolution and possibly also in human evolution.

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## Circadian Periodicity in Susceptibility to Lidocaine Hydrochloride

Abstract. *Separate groups of mice standardized in an alternating 12-hour-light, 12-hour-dark regimen were treated with lidocaine hydrochloride every 3 hours over a 24-hour period. The results indicate a quantitative circadian periodicity with maximal convulsant activity at 2100 hours which was approximately a fourteen fold increase over the values observed at 1500 hours.*

Recent studies have elucidated the pharmacologic response to drugs as affected by the circadian (about 24-hours) rhythms of animals. Mice subjected to alternating light and dark regimens, a factor known to influence their rhythmical behavior, displayed a circadian response to several pharmacologic agents, namely, pentobarbital (*1*, *2*), nikethamide (*3*), methopyrapone (*4*), and aurothioglucose (*5*). The degree of response was found to vary quantitatively along a 24-hour time scale.

The experiment reported here was conducted to determine whether mice, standardized in constant environmental conditions with alternating 12-hour-light and 12-hour-dark cycles, would display a significantly circadian quantitative response to the central stimulant activity of the local-anesthetic drug lidocaine hydrochloride. In therapeutic doses lidocaine hydrochloride inhibits the conduction of impulses along a nerve fiber. However, central nervous system stimulation, even to the point of convulsions, is the most frequent and serious systemic toxicity response induced by lidocaine hydrochloride. The existence of a direct relationship between the degree of susceptibility to convulsions induced by lidocaine hydrochloride and mammalian 24-hour rhythms could be of considerable interest to clinicians in medical, dental, and veterinary practices.

Adult female albino mice (Carworth Farms, CF-1), 22.24 ± 0.23 g body weight, were housed 16 per cage with a total floor area of 1216 in.$^2$ (7845 cm$^2$), or 7.6 in.$^2$ per mouse; this eliminated the psychological variable of aggregation due to overcrowded housing. The mice were housed in a controlled environmental room maintained at a temperature of 23.3° ± 1.0°C, with a relative humidity of 65 ± 2.0 percent. The room was programmed so as to provide 12 hours of incandescent lighting (four 40-watt bulbs) from 0605 to 1805 hours and a totally darkened phase from 1805 to 0605 hours. Testing during the dark phase was conducted in an adjacent room having similarly controlled conditions, except that in order to make observations possible a very low level of illumination was maintained by means of a 20-watt photographic safelight. During the light phase animals were tested under conditions com-

parable to those under which they were being maintained. Mice were provided a minimum of 14 days' acclimation time in the controlled environment before testing. Water and Purina mouse chow were freely available during the acclimation period.

On the basis of preliminary experiments a 65-mg/kg dose of lidocaine hydrochloride was selected as that dose of lidocaine which, over a 24-hour period, most nearly approximates a $CD_{50}$ (convulsive dose in 50 percent of the mice) and which has a latent period of 5 to 10 minutes. The latent period maximum of 10 minutes was necessary so as to limit the duration of any single testing period to no more than 30 minutes, a factor not adequately controlled in some reports in the literature.

Beginning at 1500 and at 3-hour intervals over the succeeding 24-hour period, groups of 16 mice received intraperitoneal administrations of lidocaine hydrochloride, 65 mg/kg, so diluted with 0.9 percent saline as to permit the administration of 0.01 ml of the final solution per gram of body weight of a mouse. At a later date a group of 20 mice received the same dose at 2100 hours to test the significance of a peak response at this hour. Each mouse was weighed, injected, and then placed in a separate trough of an inclined wire screen maintained at a 60° angle with the table top. Isolation on the inclined screen minimized physical contact between animals and therefore added to the precision with which the end point was determined. The end point was considered as being the inability of the mouse to maintain its position on the inclined screen as the result of the convulsant activity of the administered lidocaine hydrochloride. The methods of Snedecor (*6*) were used to perform the analyses of variance and the chi-square tests.

An analysis of variance performed on the data for percent convulsion in Table 1 established that there were no differences ($p = 0.30$) between the responses within the dark or the light phases, but that there was a statistically significant difference ($p = 0.02$) between the pooled responses of the light phase and the pooled response of the dark phase. As a point of reference for determining the statistical significance of individual time-period responses in Table 1, the mean 24-hour response to lidocaine hydrochloride (65 mg/kg) was calculated to have been 38.6 ± 8.7 percent convulsions. Chi-square analysis of each 3-hour response compared with the mean 24-hour response revealed, as did the analysis of variance, that only the maximum (2100) and the minimum (1500) response points (Table 1) were significantly different from the mean 24-hour response ($p < 0.001$, in both cases). Table 1, therefore, reveals a typical circadian response curve with a significant maximal response of 83 percent convulsions occurring at 2100, or 3 hours after the onset of the dark period. A gradual decrease in response occurred, beginning at 2400 hours, in the middle of the dark period, and continuing on a downgrade to reach a trough at 1500 hours, in the light period, at which time only 6 percent of the mice exhibited convulsions.

Table 1. Convulsant activity in mice in response to lidocaine hydrochloride (65 mg/kg, intraperitoneally). Mean daily response ± standard error of mean = 38.6 ± 8.7 percent convulsions (reference standard for individual time periods).

| Condition | Time* | Convulsions † (%) |
|---|---|---|
| Light | 1500 | 6 ($p < .001$) |
| Light | 1800 | 56 ($p = .02$) |
| Dark | 2100 | 83 ($p < .001$) |
| Dark | 2400 | 50 |
| Dark | 0300 | 44 |
| Dark | 0600 | 25 |
| Light | 0900 | 19 |
| Light | 1200 | 25 |

* Light phases: lights on, 0605 to 1805 hours; lights off, 1805 to 0605 (14–15 December 1964). † Sixteen adult female albino mice per time period, except mean of 32 mice from two 1500 and 36 mice from two 2100 time injections. Chi-square analyses performed by the methods of Snedecor (*6*).

Other drugs tested in mice, although having other end points, show similar circadian patterns of response but have their crests and troughs at different points of time during a 24-hour period. The toxic effect of the adrenal cortical inhibitor, methopyrapone (SU 4885), caused the highest death rate in mice at 2100 hours, or at close proximity to this time, depending upon the drug concentration used (*4*). The susceptibility was similar in its phase relationships to our circadian response for lidocaine. The analeptic drug, nikethamide, however, displayed a response pattern of maximum mortality in mice at 1400 hours and a low incidence at 0200 hours that was a mirror image of our curve, although slightly out of phase in its temporal relationship (*3*). Mice (CBA strain) injected with the anti-arthritic drug aurothioglucose showed approximately a 2-g weight increase and a four and one-half fold increase in mortality at 1200 hours (day) relative to 2000 hours (night) (*5*). The influence of the circadian rhythm on the duration of sleep induced by pentobarbital was the greatest at 1400 hours in mice (*1*). The pattern of circadian response to various drugs becomes quite variable as to their temporal relations even though the standardization of experimental mice in alternating light and dark periods was essentially similar in all investigations. This is reasonable to expect, since each drug has a different effect on the physiological system of an experimental animal.

The pattern of response to lidocaine shown in Table 1 presents a clear demonstration of a definite quantitative response varying along a 24-hour time period. Studies on the metabolism of lidocaine have reported that only 10 to 20 percent of this drug was excreted unchanged after administration to dogs (*7*). In man even less lidocaine was recovered in the urine (*8*). In vitro studies using albino rabbits demonstrated that only liver tissue has any appreciable capacity to metabolize this drug, a process which requires molecular oxygen and TPNH (reduced triphosphopyridine nucleotide) (*9*). Viewing our results in the light of these facts suggests that there may be varying rates of liver metabolism of this drug throughout a 24-hour period, that is, there may be a 24-hour rhythm of lidocaine metabolism by the liver. However, other related factors, such as differential rates of absorption, distribution, and membrane permeability, should not be excluded from playing a role in susceptibility to lidocaine during the 24-hour experimental period. Irrespective of these physiological factors, there appears to be the coincidence of a relationship between the motor activity of this nocturnal animal and the crests in susceptibility to convulsions after lidocaine administration. At present no plausible explanation of the factors underlying this coincidental relationship is possible, but a 24-hour periodic phenomenon is present.

From this study the circadian system of mice has been found to have a considerable influence on the susceptibility of mice to the central stimulant activity of lidocaine hydrochloride. The environmental factors of light and temperature and possibly sociopsychological conditions being the most important (*1, 10*), they must be considered as having influential effects on the circadian system of mice and should be controlled, if possible, before and dur-

ing the time in which lidocaine is administered and is active. Significance of these data to the practitioner might be that in a diurnal animal such as man, increased susceptibility to the toxicity of lidocaine hydrochloride may occur more frequently in the activity phase of the animal's circadian cycle, and consequently time of day should be considered in the determination of each individual dose of lidocaine hydrochloride.

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## Thalamic Reticular System and Central Grey: Self-Stimulation

Abstract. *Rats will press a bar that brings about stimulation of midline thalamic reticular, periventricular, and central grey brain regions. In the latter two regions stimulation can also cause pain or fear.*

There is evidence to indicate that electrical stimulation of midline and intralaminar nuclei at low frequencies in the unanesthetized animal can induce sleep (*1*). In the same kind of preparation, stimulation at higher frequencies characteristically induces stereotyped motor movements or visual searching responses (*2*). Self-stimulation mapping studies of the brain have included few electrode placements within this area (*3*, *4*). Nevertheless, on the basis of these studies investigators have suggested that self-stimulation is not characteristic of thalamic reticular system placements (*4*, *5*). It has also been concluded that self-stimulation cannot be obtained with electrodes implanted in periventricular and central grey brain regions (*4*, *6*). In contrast to these beliefs, the present study demonstrates that self-stimulation occurs with electrodes located within the thalamic reticular system, periventricular, and central grey regions of the brain.

Single, bipolar, enameled stainless-steel wire electrodes were implanted in rats, by techniques outlined previously (*7*). Each animal was assessed for self-stimulation in the Skinner box test. In this test a bar-press resulted in 60-cycle sine wave stimulation of the brain for a duration up to 0.5 second. Operant rates of responding without brain stimulation were established over four daily 15-minute sessions. On the fifth and subsequent days a bar-press closed a circuit that resulted in brain stimulation. Current levels were usually increased each day. On the basis of the Skinner box test the animals could be grouped into three classes. In the first class (SS) a current level was reached at which the animals consistently self-stimulated during the test periods. Bar-press rates ranged from 200 to 300 presses within the test period. Self-stimulation was usually accompanied by forced motor movements involving the forelimbs and neck. Observation of these animals in the Skinner box suggested that the brain stimulation had no noxious effects in spite of the fact that at high current levels the forced motor movements were sometimes intense enough to throw the animal about.

Animals in the second class (SS-P) also demonstrated self-stimulation behavior. In some cases, however, self-stimulation was obvious only after repeated test sessions at the same current level. Thus, Fig. 1 indicates no rise in response rate by an animal first tested at a particular stimulation intensity. Observation had suggested, however, that the stimulation had reached an intensity at which it was having some effect. Repeated sessions at the same intensity bore this out. Several SS-P animals exhibited this same behavior and in some instances bar-press rates rose by several hundred presses over sessions at the same stimulation intensity. Some animals in the SS-P group did show a rise in pressing rate when first tested at an effective current level. In some cases this rate was maintained or increased, while in others it was followed by a decrease, at higher current levels.

All SS-P rats as well as SS rats self-stimuated at above operant bar-press rates. Unlike SS subjects, however, self-stimulation was accompanied by signs

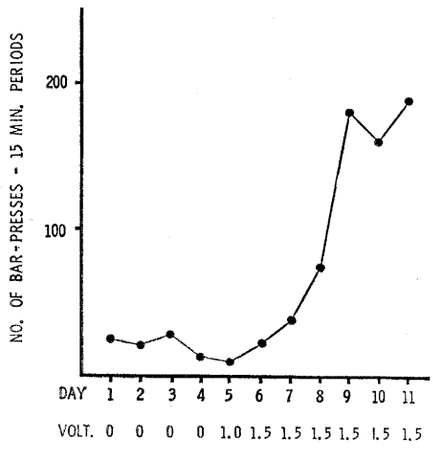


Fig. 1. Electrode in periventricular grey. Note slow rise in response rate over daily sessions at the same stimulus intensity.

of pain and fear (for example, shrieking) in SS-P animals. Usually when the shrieking reached the point where it accompanied each of a number of bar-presses the animal stopped manipulating the bar for several seconds, only to repeat the cycle again. Approach-avoidance behavior toward the bar was also noted.

Other observations also bore out the distinction between class SS and class SS-P animals. For example, many of these rats were placed in a rectangular box with a movable elevated platform. The weight of the animal on the platform closed a microswitch which initiated a train of 0.5-second-on, 0.5-second-off brain stimulation. At above-threshold currents SS animals would learn to move onto the platform and stay there. SS-P animals would move onto the platform and then off repeatedly. Limited use was made of experimenter-controlled brain stimulation. However, observation of SS animals during several seconds of on-off brain stimulation at above-threshold self-stimulation current levels did not indicate noxious effects. Such stimulation of SS-P animals resulted in running movements that were clearly indicative of pain or fear.

Most animals of our third group (U)

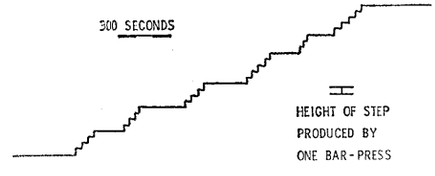


Fig. 2. Electrode in central grey. Representative portion from a prolonged 200-minute test session. Bar-press rate is low but regular, indicating self-stimulation.