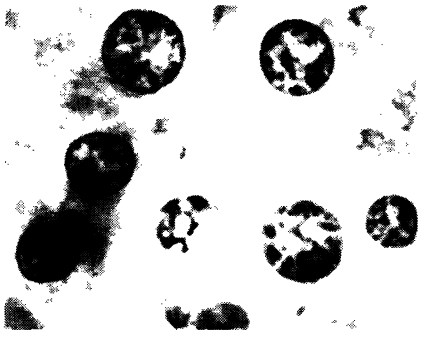

Fig. 4. Adult male pseudohermaphrodite. Interstitial cells of gonad, showing male chromatin pattern (× 1000) (Feulgen).

Obviously it is even more important to have the same guidance available in the study of aberrations at the embryonic stages. So far it appears that testis differentiation starts fairly regularly during the seventh week (about stage 30), but frequently it shows partial delays, with consequent persistence of cortical remnants and retardation of differentiation of the secondary sex organs. Transplacental interactions between male embryo and mother have been suggested as a possible cause (*10*), mainly on the basis of animal experiments (amphibian parabiosis). Recent developments, however, have opened direct approaches to this problem of human sex differentiation (*11*).

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## Operant Behavior during Sleep: a Measure of Depth of Sleep

Most animals spend approximately 30 percent of their lives asleep, yet remarkably few experimental investigations of sleep have been conducted, possibly because of the difficulty of measuring sleep. Processes that are difficult to measure may be studied in behavioral as well as in physical sciences by analyzing the frequency, duration, and degree of their interference with a more easily measured process (*1, 2*). In this report I present a method for measuring the duration and depth of sleep by recording how much it suppresses the rate of a reinforced operant response and compare the results with those obtained by measurement of body movements (*3*).

A sleep-deprived subject wearing an aviator's helmet was placed in a comfortable bed; the helmet contained an earphone through which a pure tone of 2000 cy/sec was delivered to the subject's ear. Each response (subject's thumb closing a microswitch taped into his preferred hand) was recorded on a counter and a Harvard cumulative recorder. A rate analyzer (*4*) controlled a potentiometer which reduced the intensity of the tone after each response. Rapid operation of the switch reduced the tone to zero intensity, and the subject could avoid the tone by continued responding. Slow operation of the switch kept the tone at a moderate intensity. If the switch was not operated, the tone rose to and was maintained at its full intensity (30 db). Thus the subject's rate of response controlled the intensity of the tone.

To record body movements, the base of a brass rod (9 in. long and ¼ in. in diameter) was suspended through the center of a brass washer (⅝ in. inside diameter) by a light spring from the center of the bed spring. A body movement was recorded when slight movements of the subject made the rod contact the washer.

Sleep records were taken under conditions of (i) 15 hours' sleep deprivation; (ii) 15 hours' deprivation plus 1½ grains of seconal ingested 5 minutes before retiring; (iii) 38 hours' deprivation; and (iv) 15 hours' deprivation without the tone. Since the latter condition was presented last, it provided a control for conditioned responding effects. Prior to the control session, the subjects were instructed to respond whenever they were awake at the rate they had on previous nights. Thus, behavior maintained by escaping the aversive tone could be compared with behavior maintained by recalled verbal instructions and previous conditioning. Two adult males, aged 20 and 34, served as subjects.

Figure 1 contains sample cumulative response records (selected as representative of 40 similar records) for one subject during eight continuous hours in bed on each of six different nights. Records for the first 4 hours (Fig. 1, top) show operant behavior during the deep initial

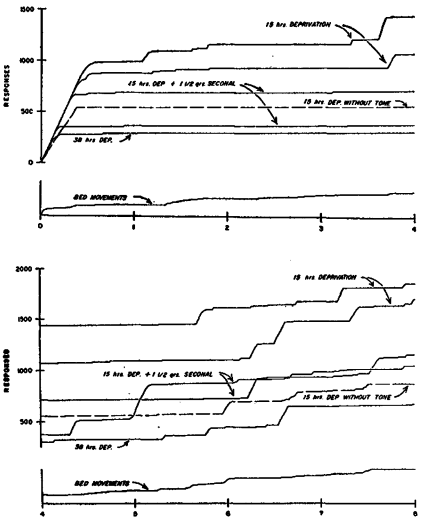

Fig. 1. Cumulative responses reinforced by a reduction in tone intensity are plotted against time in bed. (Top) First 4 hours in bed; (bottom) second 4 hours in bed. The lower the slope of the curves, the more intense was the tone and the deeper was the sleep. A cumulative record of body movements is presented at the bottom of each part.

sleep, and records for the last 4 hours (Fig. 1, bottom) show the subsequent light waking state characterized by bursts of responding. Records of short daytime naps contain response bursts very similar to those of the light waking state. The major effects of sleep deprivation and sedation on operant responding during sleep occur during the first 4 hours of sleep.

The two records for 15 hours' deprivation show the pattern of normal sleep. The subject spent 24 minutes in bed before the response rate dropped (sleep latency), and an additional 16 minutes passed before the rate dropped to zero (sleep onset). The period of deep sleep (from the time responses dropped to zero rate until 100 responses were emitted and during which the tone sounded at its full intensity) was 2 hours. Notable is the fact that approximately the same amount of responding occurred over the whole night on both 15-hour deprivation records, despite the separation of the two curves by 400 responses after 4 hours of sleep. The same effect appeared for the condition of 15 hours' deprivation plus seconal.

The addition of seconal to 15 hours' deprivation produced deep sleep sooner (sleep latency, 13 minutes) and more abruptly (sleep onset, 3 minutes) than did 15 hours' deprivation alone. Also, seconal doubled the deep sleep period (4 hours) and produced deeper sleep since fewer response bursts were emitted.

The 38-hour deprivation record was similar to the seconal record, with a short sleep latency (7 minutes) and an abrupt

sleep onset (5 minutes). The deep sleep period (5½ hours) was longer and was characterized by fewer response bursts than it was for both normal and drugged sleep conditions.

The control record showed a 23-minute sleep latency (similar to unconditioned responding) but an immediate sleep onset (0 minutes). Thus, conditioned responding did not show the gradual sleep onset characteristic of unconditioned responding. Note also that the initial conditioned response rate during the latency period (base line) was lower than the unconditioned rate, showing inaccurate recall. The deep sleep period was longer (5¾ hours), and the rate of response during deep sleep was lower for conditioned responding than for unconditioned responding.

The records of body movements did not show the sleep-latency or sleep-onset differences for the conditions of deprivation and sedation that were shown by operant responding. Fewer movements were made during deep sleep than during the later waking state for all conditions, however, and therefore the method could show that deprivation and drugs increase the duration of deep sleep. This effect has been reported previously (*5*).

The subjects' reports of the number of times they recalled awakening were not related to the number of response bursts in the sleep records. Neither subject reported ill effects of the experiment, and both felt rested after the sessions.

These observations show that unconditioned operant responding to turn off an aversive stimulus during sleep is more sensitive to intermediate sleep levels and to deprivation and drug effects than is responding supported by verbal instructions with previous conditioning or the recording of body movements. This sensitive and widely applicable method should enable scientists to study sleep behavior more effectively. It can be used to investigate the effects of drugs, neurosurgery, deprivation, and awakening stimuli on the sleep of lower animals as well as on that of human beings. Records of operant responding during sleep and hypnosis should be compared with electroencephalographic records in normal and abnormal subjects.

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11 October 1957

## Surface Ionization of Silver; Silver in Meteorites

Isotopic dilution followed by mass spectrometric analysis has been applied to many of the naturally occurring elements (*1*). Concentrations may be accurately determined at the level of one part per million (ppm) and even lower. Surface ionization of a solid sample is the usual source of ions for the heavier elements. A recent study (*2*) of microgram amounts of lead, uranium, and thorium from typical rock minerals emphasizes the importance of these sensitive techniques to geochemistry. The experiments described in this report (*3*) demonstrate that silver may be similarly analyzed.

These techniques may lead to a better understanding of the distribution of silver in the earth and in meteorites. The analysis of small amounts in meteorites is especially important for the following reasons.

1) A recent estimate (*4*) places the cosmic abundance of silver at 0.26 atom per million atoms of silicon. This value is based on a reasonable interpolation from elements whose abundances are better established. A typical earlier estimate (*5*) is an order of magnitude higher. In this case, the ratio of 2.7 atoms of Ag per million of Si is based on analyses of meteoritic phases by the Noddacks. There are several indications that their concentration may be too large (*6*).

2) The isotopic composition of silver may reveal part of the early history of the solar system. If processes leading to the formation of the planets occurred shortly after nucleogenesis, variations in the relative amounts of $Ag^{107}$ and $Ag^{109}$ may exist as a result of the decay of $Pd^{107}$. The half-life of this extinct nuclide is reported (*7*) as $7.5 \times 10^{-6}$ years. Even with very favorable fractionation of Ag relative to Pd, the detection of such an effect would imply that the period between nucleogenesis and the formation of the earth was considerably shorter than the minimum time estimated on the basis of the $17.2 \times 10^{-6}$-year half-life of $I^{129}$ (*8*).

Although positive results from a search for isotopic differences in silver appear to be unlikely, a sample of troilite has been investigated. This material was selected initially because of its relatively high concentration of Ag. It was hoped that the troilite either might have scavenged $Ag^{107}$ from the surrounding palladium-rich (*9*) metallic phase or might have remained isolated from an early stage and thus retained primeval silver.

Troilite from the Xiquipilco (Toluca) iron meteorite, a medium octahedrite, was received in the form of slabs several millimeters thick and several centimeters in breadth (*10*). The surface was ground off with an $Al_2O_3$ refractory wheel (dental size). The pieces were rinsed several times with $2N$ $H_2SO_4$ (*11*) and quadruply distilled water.

The dried sample, weighing 18.40 g, was dissolved, except for a black residue, in two stages requiring nearly 20 ml of concentrated $H_2SO_4$ in about 250 ml of water. The residue was centrifuged away from most of the iron and nickel, washed, and digested in quartz-distilled concentrated $HNO_3$. This solution of about 30 ml was mixed with 30 ml of a 20 percent solution of purified ammonium citrate. The *p*H was adjusted to slightly greater than 1 with $NH_4OH$ (carefully prepared from gaseous $NH_3$ and distilled water). Successive 2- to 3-ml portions of a dithizone solution (*12*) were briefly shaken with the acid solution until they became violet after shaking instead of yellow (silver dithizonate). The $CHCl_3$ was evaporated, and the residue was taken to dryness several times with concentrated $HNO_3$. The residue was taken up with a few drops of concentrated $HNO_3$, diluted to about 25 ml, and the *p*H was adjusted to between 1 and 1.5 with $NH_4OH$. Extraction with standard dithizone solution in 2-ml portions required 20 ml and was equivalent to 30 μg of silver. This is only an upper limit for the amount of Ag, for Hg is also extracted under these conditions. The dithizonate was again converted to nitrate (*13*).

A chunk of Canyon Diablo iron meteorite, free of visually obvious troilite inclusions, was rinsed four times with $6N$ $H_2SO_4$ and washed with distilled water after each acid rinse. The dried weight of this piece was 102.6 g. It was almost completely dissolved in 150 ml of concentrated $H_2SO_4$ and 550 ml of distilled water. The odor of $H_2S$ was present during the reaction. Further treatment of the residue required approximately an additional 40 ml of concentrated $H_2SO_4$ and roughly 700 ml more water. Gaseous $H_2S$ was also added shortly before separation of most of the iron and nickel in the supernatant from the residue. The final residue consisted of sulfur, black particles, and a small amount of heavier, metallic slivers about 1 mm long.

The solids were digested with about 150 ml of concentrated $HNO_3$. The resulting 25 ml of solution were mixed with 25 ml of the ammonium citrate solution, adjusted to a *p*H of 1.5, and extracted