Effect of LSD on the Sleep Cycle of the Developing Kitten

AKIRA SHIMIZU
HAROLD E. HIMWICH

Thudichum Psychiatric Research Laboratory
Galesburg State Research Hospital
Galesburg, Illinois

SHIMIZU, AKIRA, and HIMWICH, HAROLD E. (1968). *Effect of LSD-25 on the Sleep Cycle of the Developing Kitten.* DEVELOPMENTAL PSYCHOBIOLOGY, 1(1): 60–64. The effects of LSD on the sleep cycle were studied in 44 kittens during the growth period of 1 to 28 days of age. Electroencephalograms, electromyograms of the posterior neck muscles, and the respiratory rhythm were recorded and gross behavior was observed. With 10 μg/kg of *d*-lysergic acid diethylamide, the per cent time of activated sleep in total sleeping time was diminished. This diminution was less marked in kittens 6 to 8 days of age or younger and became more significant in the kittens 11 to 13 days or older. With 20 μg/kg the decrease of per cent time of activated sleep was highly significant at all ages. With a larger dose, the influence of age could no longer be demonstrated. The duration of single episodes of activated sleep showed some evidence of shortening, but this effect was also not related to age.

lysergic acid diethylamide REM sleep electroencephalogram sleep cycle, kittens electromyogram, neck muscles development

THE EFFECTS of *d*-lysergic acid diethylamide (LSD-25) or its congeners on behavior and the electroencephalogram (EEG) (Hoch, Pennes, & Cattell, 1953; Abramson, Jarvik, Kaufman, Kornetsky, Levine, & Wagner, 1955; Rinaldi & Himwich, 1955; Hirsch, Jarvik, & Abramson, 1956; Solms, 1956; von Felsinger, Lasagna, & Beecher, 1956; Monroe, Heath, Mickle, & Llewellyn, 1957; Bradley, 1958; Isbell, 1959; Schweigerdt, Stewart, & Himwich, 1966) and on the sleep cycle in both man (Green, 1965; Muzio, Roffwarg, & Kaufman, 1968) and animals (Hobson, 1964; Khazan & Sawyer, 1964) have been investigated extensively. Since LSD is hallucinogenic, researchers have anticipated that it would increase dreaming time for man as expressed in "activated" or "rapid eye movement" (REM) sleep. This expected increase in activated sleep time occurred in the study by Green (1965). Muzio *et al.* (1968) observed that either the first or second activated sleep episode of the experimental night was prolonged, though this prolongation ceased thereafter and the total activated sleep time for a given night did not increase further. On the other hand, Hobson (1964) has noted reductions in the proportion of activated sleep to total sleep time in the cat and suggested that LSD antagonizes the system mediating activated sleep. A similar suppression also was demonstrated in the rabbit (Khazan & Sawyer, 1964). The apparent discrepancy in the results between humans and experimental animals can be attributed to differences in dosage, rather than to species differences. In the experimental animals, more than 20 μg/kg was used when the significant decreases occurred in the per cent time of activated sleep, whereas much smaller doses were given to the human subjects. All of these studies concerning the effect of LSD on the sleep cycle have been carried out in adult organisms; until now, no study has been performed on very young subjects.

Since the sleep-wakefulness cycle of kittens develops rapidly during the first postnatal month (Jouvet, Valatx, & Jouvet, 1961; Valatx, Jouvet, & Jouvet, 1964; Shimizu & Himwich, 1968), it was expected that the effect of LSD on the sleep cycle might change during growth. The present study was undertaken to clarify the effect of LSD on the sleep cycle of kittens during postnatal development.

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METHOD

SUBJECTS

Forty-four growing kittens of both sexes were studied from the first day after birth until 28 days of age. They appeared normal at birth and developed thereafter in a satisfactory manner, exhibiting appropriate increases in body weight.

PROCEDURE

On an experimental day, a kitten was taken from the mother shortly after nursing and placed in a warm, glass cylinder, measuring 30×30 cm, in a quiet, dimly lighted room. The EEG, electromyogram (EMG) of posterior neck muscles, and respiratory rhythm were recorded on a Grass IIID electroencephalograph while observations of gross behavior were made. Small nickel-silver skin clips were used as electrodes. For the EEG recordings, two clips were attached to the skin over the scalp: one on the left side, anterior to the coronal suture, the other on the right, posterior to the coronal suture. A clip on the upper neck served as reference electrode. The EMG activity of posterior neck muscles was monitored through two clips passed through the skin and attached to the muscles. All clips were fixed in place 20 min before beginning the recording. Respiratory rhythm was monitored by means of a rubber tube containing copper-sulfate solution which was placed around the thorax. Changes in length, hence resistance of the copper-sulfate tube, were recorded on an electroencephalograph (Grass IIID) through a circuit containing a resistance and a battery. A solution of LSD was given through a polyethylene stomach tube at two dose levels: 10 and 20 μg/kg. The amount of the solution was between 0.3 and 0.8 cc depending upon the age of the animal. In the pilot study, saline in these amounts did not affect the parameters under investigation. Kittens were given LSD 5 days or more after saline administration. The polygraphic recordings started 50 min after drug administration and were continued consistently for periods of 80 to 100 min.

DATA ANALYSIS

Each page of the record was of 20-sec duration and was judged as mainly presenting either wakefulness, slow-wave sleep (in neonates, this is called calm sleep), or activated sleep (in neonates, sleep-with-jerks). From these data, we calculated (*1*) total sleep time, (*2*) per cent time of activated sleep to total sleep as the ratio of the number of pages of the record representing activated sleep to the number of pages of total sleep. Since total sleeping time is the sum of activated sleep time and slow-wave sleep time, per cent time of slow-wave sleep can be calculated by subtracting the per cent time of activated sleep from 100. (*3*) The mean durations of single episodes of slow-wave sleep and of activated sleep also were calculated. The effects of the drug on these parameters were analyzed in relation to age.

At first, we also calculated the per cent time of wakefulness in the total recording time. However, the LSD-treated kittens, especially those older than 16 to 18 days of age, became very sensitive to stimuli from the environment, especially to auditory stimuli as noted by Bradley (1958) in the adult cat. Thus the per cent time of wakefulness after LSD differed substantially from one experiment to another in the same age group depending upon the amount of environmental stimulation they received. For this reason, the per cent time of wakefulness was not calculated. Moreover, since sustained arousal can change the per cent time of activated sleep in total sleep (Ferguson & Dement, 1967), the kittens which did not sleep for more than 60% of the total recording time were omitted in our calculation.

RESULTS

DEVELOPMENT OF SLEEP CYCLE IN THE CONTROL KITTENS

As reported previously (Jouvet *et al.*, 1961; Valatx *et al.*, 1964; Shimizu & Himwich, 1968), sleep patterns of the adult type were observed after the kittens were 13 to 15 days of age. However, two phases of sleep could be differentiated in the kittens younger than these ages: calm sleep and sleep-with-jerks, respectively. Calm sleep changed gradually into slow-wave sleep and sleep-with-jerks into activated sleep before they were 13 to 15 days of age (Shimizu & Himwich, 1968). The criteria for judging whether a kitten is in wakefulness, calm sleep, or sleep-with-jerks are described elsewhere (Shimizu & Himvich, 1968) and may be summarized as follows: (*1*) *Wakefulness:* The kitten was either moving its head, crawling, or crying with considerable tonic EMG activity of the posterior neck muscles; (*2*) *Calm Sleep:* The kitten was lying quietly and activity of the posterior neck muscles, as recorded in the EMG, was either absent or decreased as compared to a state of wakefulness. Respiratory rhythm was generally slowed in comparison with that during wakefulness, but occurred regularly; (*3*) *Sleep-with-Jerks:* The kitten was lying relaxed but with very rapid jerking movements of the head, limbs, toes, tail, eyelids, whiskers, and sometimes of the whole body. Tonic EMG activity of the posterior neck was absent and respiratory rhythm was very irregular during this sleep phase. However, no clear differences were found in the EEG patterns that would enable the experi-

TABLE 1. *Effect of LSD on Per Cent Time of Total Sleep in "Activated" Sleep* [a]

Age of kittens (days)	Control (% time)	LSD dosage 10 μg/kg (% time)	LSD dosage 20 μg/kg (% time)
1–3	88.0 ± 2.8	73.8 ± 4.5 [b]	67.1 ± 3.8 [c]
6–8	74.0 ± 3.7	64.1 ± 3.3 [b]	54.9 ± 7.3 [c]
11–13	68.0 ± 4.6	52.1 ± 7.7 [c]	48.9 ± 7.7 [c]
16–18	61.9 ± 5.8	46.7 ± 6.7 [c]	44.1 ± 6.0 [c]
21–23	54.4 ± 5.9	34.5 ± 7.5 [c]	31.4 ± 9.9 [c]
26–28	45.3 ± 7.2	29.9 ± 5.5 [c]	27.8 ± 7.6 [c]

[a] Percentages are mean values ± S.D. For each age group, control value was based on an N of 7; values after LSD treatment were based on an N of 4 at each dose level. Levels of significance are based on comparisons between control and LSD-treated kittens in the same age group. The Student t-test was used.

[b] $p < .025$.

[c] $p < .005$.

menter to distinguish between the three phases of the sleep-wakefulness cycle in kittens 1 to 12 days of age.

As shown in Table 1, activated sleep (*i.e.*, sleep-with-jerks) occupied about 90% of the total sleeping time in the control kittens at 1 to 3 days of age, but this percentage diminished with increasing age. The mean durations of single episodes of both slow-wave sleep and activated sleep increased with age (Table 2).

EFFECTS OF LSD

Per cent time of activated sleep in total sleeping time was reduced at all ages following administration of 10 μg/kg of LSD (Table 1). Accordingly, the amount of slow-wave sleep increased. The diminution of per cent time of activated sleep in these kittens was of lesser significance through 6 to 8 days of age ($.01 < p < .025$); at 11 to 13 days of age and older, it became highly significant ($p < .005$). When the dose of LSD was increased from 10 to 20 μg/kg, the diminution of per cent time of activated sleep became more marked at each age level, through effects at the two different dosage levels in the same age groups were not compared statistically. At 20 μg/kg, the diminution was highly significant at all ages ($p < .005$), compared to the control values, and an influence of age was not observed.

The mean durations of single episodes of slow-wave sleep were not affected by 10 μg/kg of LSD (Table 2). With 20 μg/kg, the durations of this parameter showed a numerical tendency to shorten, but this was not statistically significant. On the other hand, the mean durations of single episodes of activated sleep became shorter with 10 μg/kg, and were statistically significant at 11 to 13 and 16 to 18 days of age, but not in any other age group (Table 2). With 20 μg/kg, the shortening of the mean durations of single episodes of activated sleep was significant only in kittens 11 to 13 days of age, or older (Table 2).

Generally speaking, then, the LSD-treated kittens were in activated sleep a smaller percentage of total sleep time and had shorter durations of single episodes of activated sleep, in comparison with control kittens of the same age. However, the jerking movements, or sleep-with-jerks, of LSD-treated kittens during activated sleep tended to occur in closer repetition than those of the control kittens (Fig. 1). On the other hand, no qualitative changes in slow-wave sleep or calm-sleep patterns were noted.

DISCUSSION

The present study shows that LSD diminishes activated sleep in the kitten. This is in agreement with the result of Hobson (1964) for the adult cat. At a dose level of 20 μg/kg, the diminution of percent time of activated sleep was highly significant at all ages and an influence of age was not noted. However, at 10 μg/kg, the diminution was of lesser significance in the kittens 6 to 8 days of age and younger than in older ones. A similar phenomenon was observed in

TABLE 2. *Effect of LSD on the Mean Durations of Single Episodes of "Activated" vs. Slow-wave Sleep* [a]

Age of kittens (days)	Slow-wave sleep (min) Control	Slow-wave sleep (min) LSD 10 (μg/kg)	Slow-wave sleep (min) LSD 20 (μg/kg)	"Activated" sleep (min) Control	"Activated" sleep (min) LSD 10 (μg/kg)	"Activated" sleep (min) LSD 20 (μg/kg)
1–3	0.6 ± 0.2	0.8 ± 0.2	0.8 ± 0.3	2.4 ± 0.3	1.9 ± 0.5	1.9 ± 0.7
6–8	1.2 ± 0.3	1.3 ± 0.3	1.0 ± 0.2	3.1 ± 0.6	2.6 ± 0.8	2.1 ± 0.9
11–13	1.6 ± 0.3	1.5 ± 0.3	1.1 ± 0.4	4.4 ± 0.5	2.4 ± 0.6 [b]	2.7 ± 0.5 [b]
16–18	2.3 ± 0.6	2.7 ± 0.6	2.0 ± 0.5	5.2 ± 1.3	2.9 ± 0.9 [b]	2.9 ± 1.0 [b]
21–23	3.2 ± 0.6	2.8 ± 0.6	2.6 ± 0.5	5.5 ± 1.5	3.5 ± 1.1	2.6 ± 0.9 [b]
26–28	4.7 ± 1.2	5.3 ± 1.4	3.4 ± 1.0	6.4 ± 1.4	4.7 ± 1.5	3.6 ± 1.5 [b]

[a] Figures for sleep duration are means ± S.D. For each age group, control value was calculated from the results of 7 kittens and the value after LSD was from 4 kittens at each dose level. Level of significance is based on comparisons between control and LSD-treated kittens in the same age group. The Student t-test was used.

[b] $p < .05$.

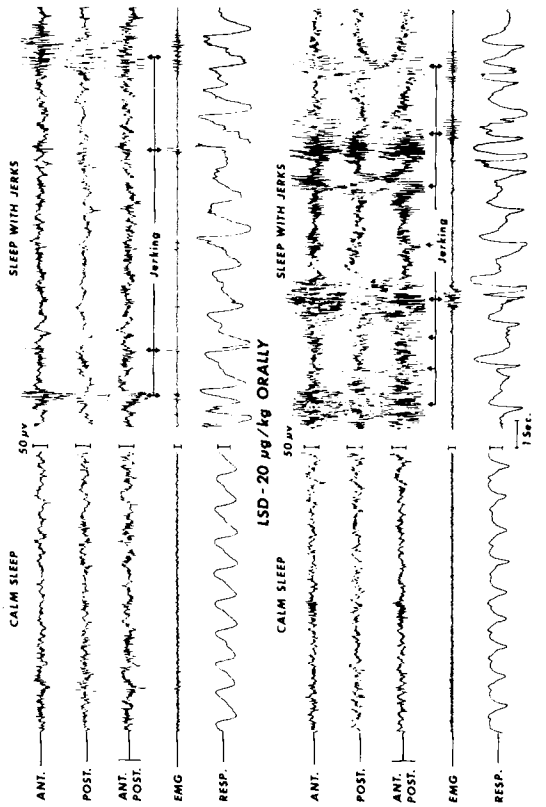

FIG. 1. Effects of LSD on sleep patterns of 5-day-old kitten. *Control:* No clear differences in EEG patterns between calm sleep and sleep-with-jerks. Tonic EMG activities of posterior neck muscles were present but with low amplitude in calm sleep. Note the jerking movements, irregular respiration, and complete loss of EMG activity during sleep-with-jerks. *LSD 20 μg/kg orally:* Note that the jerking movements during sleep-with-jerks occur in closer repetition as compared to those in the control. Ant.: anterior cortical EEG; Post.: posterior cortical EEG; Ant./Post.: bipolar tracing between two cortical areas; EMG: activity of posterior neck muscles.

our previous study on the phenothiazines, imipramine, and haloperidol (Shimizu & Himwich, 1968). These drugs also decreased the per cent time of activated sleep in the kitten, but the decrease was least marked in the neonate and became more marked as the kitten grew. The reason why the effect of these drugs on activated sleep was less marked in the newborn is not clear. The possible difference in the absorption, excretion, or metabolic rate of LSD between the adult and the newborn may be responsible. Another explanation is that the structures responsible for activated sleep are not so mature as to respond to the drug in a way similar to that of the adult. Such an example is found in the literature concerning the effects of drugs on arousal: the sympathomimetic amines and amphetamines failed to evoke an arousal reaction until the newborn kittens were 2 weeks of age (Marley & Key, 1963). Ontogenetic studies of sleep in kittens show that the mechanism responsible for activated sleep is almost functional at birth (Jouvet *et al.*, 1961; Valatx *et al.*, 1964; Jouvet, 1967; Shimizu & Himwich, 1968). Thus it may be suggested that the effect of LSD on activated sleep is qualitatively similar but less marked in intensity in the newborn kitten, compared to the adult cat, because the structures responsible for this sleep phase, while operating at birth, require several days to be established in order to respond to the drug with an intensity similar to that of the adult. The mean durations of single episodes of activated sleep tended to decrease, but this effect did not seem to be related to age (Table 2).

It has been reported that the incidence of REM during activated sleep decreased in the LSD-treated cat (Hobson, 1964) and man (Muzio *et al.*, 1968). In our study, eye movement was not recorded. However, the jerking movements which usually occur simultaneously with eye movements during activated sleep in the kitten occurred in closer repetition in the LSD-treated animal than in the control.

NOTE

Mailing address: Harold E. Himwich, Galesburg State Research Hospital, Galesburg, Illinois 61401, U.S.A.

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