Electroenceph.clin.Neurophysiol. 17, 52-56 (1964). LSD 1224

THE EFFECT OF LSD ON THE SLEEP CYCLE OF THE CAT

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(Accepted for publication: September 11, 1963)

Dement (1958) has described three distinct phases of behavior and concomitant EEG recording in the cat: waking with low voltage 5–8 c/sec waves and muscle artifact; sleep with high voltage 1–3 c/sec waves and spindles (slow wave sleep, hereafter called SWS); and sleep with low voltage 20–40 c/sec waves and rapid eye movements (low voltage fast sleep, hereafter called LVFS). When the cat's sleep was recorded after 1–3 days of forced waking, there was a regular cyclical alternation of SWS and LVFS; each pattern had a mean duration of 10 min, and the full cycle had a mean period duration of 20 min. Awakenings usually occurred during LVFS after one to five such cycles. Jouvet *et al.* (1959) confirmed these findings, and further differentiated SWS and LVFS with the observation of a relative lack of postural tone and electromyographic activity in the latter state. Jouvet (1962) has presented evidence suggesting that the mechanism responsible for LVFS in the cat is located in the pons, and that this mechanism has a cholinergic mediation.

Aserinsky and Kleitman (1955) and Dement and Kleitman (1957) described a similar cycle in human sleep with reports of dreaming following awakenings from the phase of sleep characterized by low voltage fast EEG and rapid eye movement. The psychological similarity of dreaming and the hallucinoid state associated with ingestion of *d*-lysergic acid diethylamide (LSD) (Stoll 1947) suggested the possibility of a common physiological basis for the two phenomena. If that were so and if LVFS were physiologically homologous in humans and in cats, LSD would be expected to increase LVFS in the cat whether or not dreaming or hallucinations occurred in that species.

Sympathomimetic drugs are known to oppose sleep generally, but their effects upon the sleep cycle of the cat have not previously been investigated. Cortical desynchronization and behavioral arousal are produced in waking cats by moderate doses both of *d*-amphetamine, which directly enhances reticular activation, and of LSD, which may indirectly lower the threshold to arousal via collateral afferent pathways (Bradley 1953). The cortical desynchronization that occurs within sleep and is associated with inhibition of the reticular activating system (Jouvet 1962) might therefore be produced by LSD in sleeping cats.

The following experiments were designed to study the action of LSD upon the sleep cycle of the cat, and to test the hypothesis that moderate doses would interfere with sleep generally but that small doses would selectively augment LVFS.

METHODS

Eight adult cats weighing between 1.5 and 4.5 kg were studied. Steel electrodes were implanted under pentobarbital anaesthesia by the technique of Sheatz (1961). Two electrodes were placed with their tips intracranial and extradural through separate burr holes 1 cm apart on a line parallel to, and 1 cm to the left of, the sagittal suture. Electrodes were also placed with their tips in the left and right nuchal musculature and in the left and right orbits. At least 1 week later, bipolar EEG, electromyogram (EMG), and electro-oculogram (EOG) were recorded on a Grass Model III inkwriting oscillograph at a paper speed of 0.5 cm/sec.

At 5 PM on 1 or more days prior to recording, the cat was placed on a cylindrical (3 feet in diameter) treadmill which rotated at a rate of 30 turns per hour, and exercised continuously until 8 AM when it was returned to its cage for feeding. At 9 AM the animal was weighed, in-

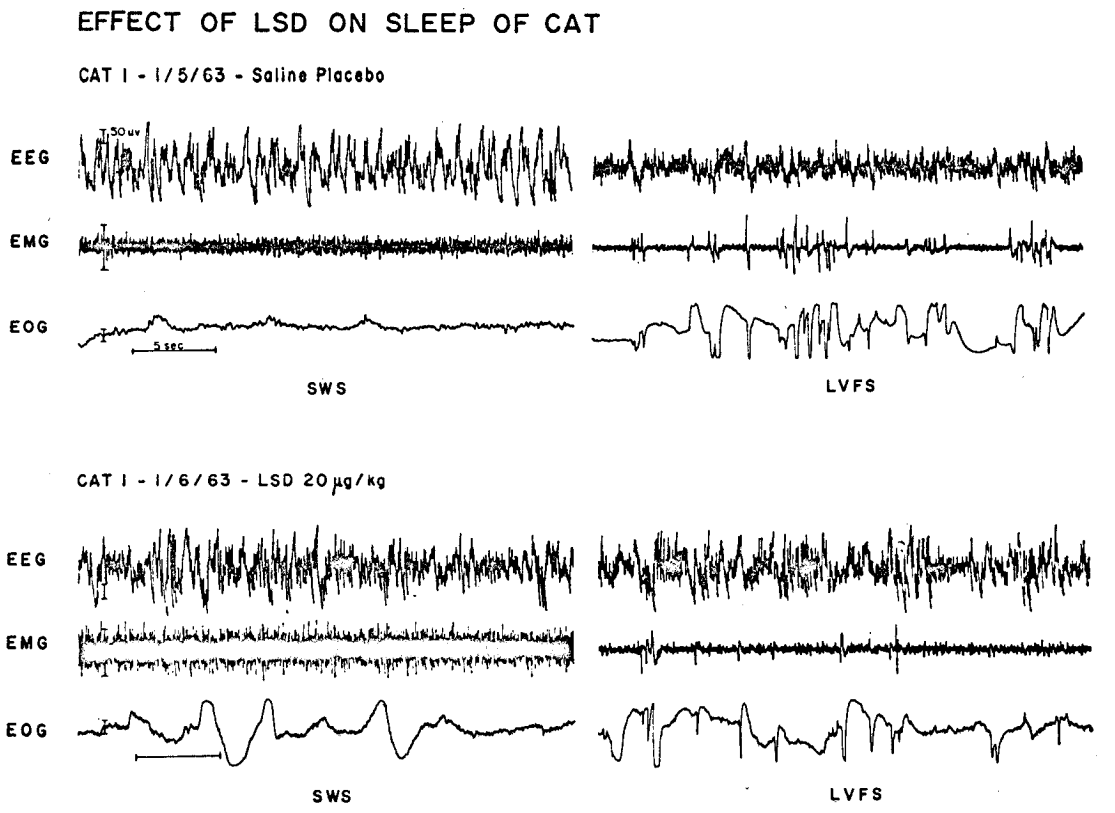

Fig. 1

Electrographic tracings. EEG, EMG, and EOG in SWS and LVFS under control conditions (upper tracings) and with 20 µg/kg LSD (lower tracings). Note the loss of EEG differentiation between SWS and LVFS with LSD. Calibration: 50 µV, 5 sec.

jected intraperitoneally with drug (see dosage in Results) or saline placebo, and placed in a soundproof, dimly illuminated box for 2 or 4 hours of continuous electrographic recording and observation from the darkened experimental room via a one-way window.

The experimenter noted the times of onset of (1) SWS (behavioral quiescence, EEG slow waves, gradual decrease in EMG amplitude, and EOG slow waves), (2) LVFS (loss of postural tone, low voltage fast EEG, sharp and pronounced decrease in EMG amplitude, and sporadic high voltage fast bursts of EOG activity) and termination thereof, and (3) electrographic arousals (low voltage fast EEG and increase in EMG amplitude) and behavioral awakenings (eyes open and looking or walking in cage). From these data were computed: (1) total sleep time, (2) duration of each full cycle (from onset of SWS to end of LVFS activity or behavioral awakening not followed by resumption of LVFS within 1 min), (3) duration of each LVFS phase, (4) the percentage of sleep time occupied by LVFS, and (5) the incidence of EEG arousals and duration of behavioral awakenings. All data were analysed for at least two and, in experiment 1, for 4 h of recording.

RESULTS

Generally, the cats given LSD went to sleep, with the appearance of EEG slow waves within about 10 min; after another 10 min, considerable admixture of lower voltage fast EEG activity was noted with either no change, or enhancement, in EMG activity (see Fig. 1) in contrast to the placebo condition. Although not systematically tested, susceptibility to arousal by incidental external stimuli was clearly increased and awaken-

ings also occurred, apparently spontaneously, during periods of slow wave sleep which seemed to be evolving toward LVFS. When LVFS occurred, it was poorly differentiated from SWS, with high voltage spindling and slow wave activity in the EEG, less pronounced falls of EMG activity, and diminished frequency and intensity of EOG activity. With LSD, arousals occurred earlier and more often during LVFS, and the duration of this phase was generally shorter than when placebo was given. The drug effect was at a maximum during the second hours, and declined but was still present during the remainder of the 4 h observation period; it was most pronounced at the highest dose levels, and barely discernible at the lowest dose levels.

Experiment 1: Each of two cats received, in a balanced order, one dose each of 2 or 20 μg/kg body weight of LSD on the 4th and 7th day of a 9 day schedule of activity and recording. The drug effect upon the percent of LVFS is seen in Fig. 2 which shows the mean percent of LVFS for the two cats. In three of the four drug trials, a rather prominent decrease was noted (see Table I), whereas in the fourth a moderate increase was seen (Cat 1, 2 μg/kg, Day 7). When the mean percent LVFS of the pooled control data was compared with the pooled data from each drug dose level, only the difference between the control and LSD 20 mean was significant statistically (two tailed *t*-test, $p < 0.005$).[1]

There was no consistent or significant effect upon either the total time awake or the number of arousals, but each tended to increase with LSD. In Cat 1 there were more brief arousals (6.5 *vs* 4.0 per 2 h), and in Cat 2 more full awakenings (22.5 *vs* 12.0 min awake) under the drug condition as compared with control. The time of sleep onset was not significantly shorter (6.75 *vs* 7.15 min), but the latency to onset of the first sustained (> 1 min) LVFS phase was more than doubled (55.0 *vs* 22.4 min) after LSD.

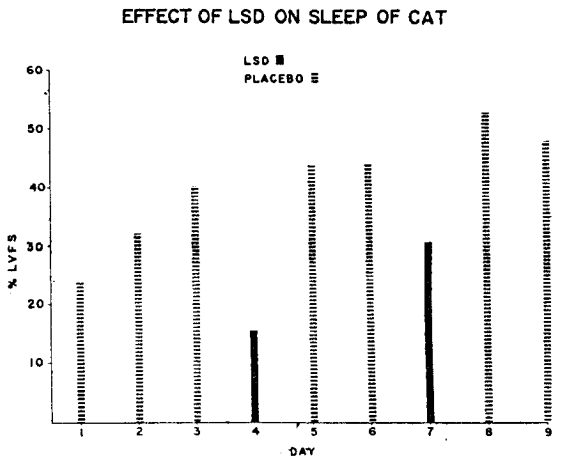

Fig. 2
Experiment 1. The reduction of percentage LVFS by LSD (solid bars) in two cats on an induced rest-activity schedule. (Each bar represents the mean result of two trials; the doses of LSD were 20 and 2 μg/kg on Day 4 and 2 and 20 μg/kg on Day 7 for Cats 1 and 2 respectively.) Note the increase in percentage LVFS on successive control days (hatched bars).

[1] Fig. 2 also illustrates the nearly stepwise serial increase in the percent LVFS on successive experimental days; the rank order correlation (R_s) of this effect was greater than 0.7 and statistically significant ($p<0.05$) for both cats with or without the drug data.

When the drug data were pooled and compared with those of the 4 control days prior to drug administration (to control for the serial effect noted above), a nearly 50 per cent reduction (42.5 to 22.5 per cent LVFS) was seen with LSD. This difference is not quite significant statistically (two tailed *t*-test, $0.1 > p > 0.05$).

Mean cycle length was not consistently altered, the pooled drug mean differing slightly and non-significantly from the control (20.9 *vs* 19.6 min); LSD, 20 μg/kg reduced that parameter non-significantly (from 19.6 to 17.8 min), while LSD 2 μg/kg increased it significantly (from 19.6 to 23.9 min).

TABLE I
Results of experiment one

Day		1	2	3	4 (LSD)	5	6	7 (LSD)	8	9
Percentage LVFS	Cat 1	20.3	31.0	40.5	15.1 (20 μg/kg)	32.0	39.7	53.9 (2 μg/kg)	43.7	49.2
	Cat 2	27.0	32.8	40.2	15.3 (2 μg/kg)	55.6	47.5	7.0 (20 μg/kg)	62.8	46.0

Effect of LSD on percent LVFS. On Days 4 and 7 the cats were given the doses of LSD indicated in parentheses.

The mean duration of the LVFS phase was generally diminished with LSD: the pooled drug mean was 4.7 min as against 7.9 min for the controls, a difference which is highly significant statistically ($p < 0.001$). This difference is contributed entirely by LSD 20 μg/kg (2.1 *vs* 7.9 min) since LSD 2 μg/kg was associated with a small but non-significant increase (8.1 *vs* 7.9 min) in mean duration of LVFS.

The observation of an increase in LVFS in one cat with 2 μg/kg in Experiment 1 suggested that the second hypothesis might be substantiated at even lower doses of LSD, but this was not borne out by similar experiments performed on the same two cats with administration of 1 μg/kg of LSD.

Experiment 2: To establish more firmly the effect of low dose levels and to permit generalization, six additional cats were studied with placebo and 2 μg/kg LSD each, after two nights of treadmill activity, with the treatment order alternated in successive animals, and with at least 1 week between trials. A reduction of all parameters was noted under the drug condition (see Fig. 3): percent LVFS was reduced from a mean of 37.1 to 17.1; five of the six animals showed this effect, which was statistically significant (paired $t = 2.42$, $p < 0.05$); LVFS mean duration was significantly decreased from 7.9 to 3.3 min, and cycle duration was reduced from 21.3 to 18.7 min. There was a lower mean percent LVFS on second trials, but the order effect was not statistically significant.

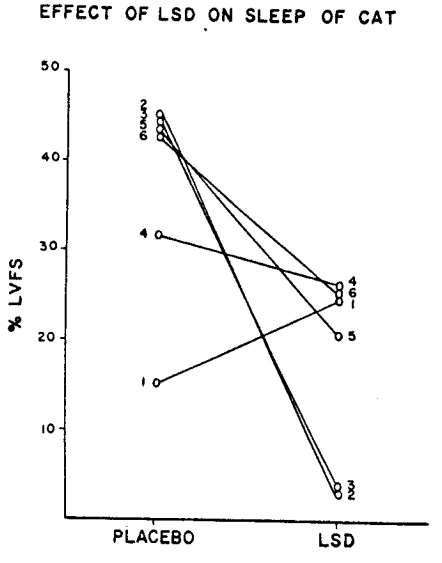

Fig. 3
Experiment 2. The reduction of LVFS by 2 μg/kg LSD in five of six cats studied after 2 days of exercise. Placebo and drug trials occurred at least 1 week apart and in balanced order.

DISCUSSION

The reduction of LVFS by LSD is most parsimoniously explained by a general interference with sleep through the production of non-specific arousal. At the behavioral level this effect is manifest in increased frequency of wakenings which does not significantly reduce the amount of time the animal is asleep but which interrupts the sustained periods of relaxation normally seen prior to the development of LVFS.

Electrographically, there is less high voltage slow wave activity during SWS, and more intermittent low voltage fast activity; the latter is often sustained as arousal, with an increase in muscle tone and an occasional full awakening which occurs especially during the period of transition between altered SWS and LVFS. When LVFS develops, however, it is less distinct, especially with reference to EEG and EOG, suggesting either a more specific, or a more intense antagonism, to the processes mediating LVFS than those mediating SWS.

The data do not contribute to an understanding of the specific mechanisms of either SWS or LVFS; whatever the origin or mediation of SWS, LSD opposes its normal development. The animals with LSD seem to be more sensitive to auditory arousal; this observation is in keeping with the evidence of Bradley (1957), and partially against that of Brown (1961) who reported a decreased threshold to visual alerting but an increased threshold to auditory alerting.

The findings are also indirectly compatible with Jouvet's evidence (1962) for a cholinergic mediation of LVFS in that an adrenergic agent is particularly potent in suppressing this phase of sleep. The mechanism for this effect was not clarified by the current experiments, but it could be qualitatively related to that proposed for SWS. The differential suppression of LVFS suggests that it is a particularly sensitive phase of sleep behavior requiring intense inhibition of

the reticular activating system and other afferent (collateral) arousal systems for the production of its particular kind of cortical activation without behavioral arousal.

The experiments clearly refute the hypothesis that LSD enhances the production of LVFS and particularly the component of cortical activation. Within the LVFS that was observed with LSD, the tendency was rather toward the synchronization which has been reported in the higher doses of LSD (Passouant 1957).

The incidental finding of significant increases in LVFS on successive days of the rest-activity schedule in Experiment 1 points to the operation of several possible variables. Adaptation to the experimental condition has been suggested by Dement (1958) to account for this phenomenon; fatigue and/or sleep deprivation resulting from the imposed activity may also play a role. Further experiments that could discriminate between these factors might provide insight into the biological significance of the sleep cycle. The magnitude of the changes in the temporal dimensions of the sleep cycle renders any concept of "normal" sleep behavior in the cat strictly relative to preceding waking activity, and probably accounts for the wide range of these dimensions reported in the literature.

In relation to clinical studies, the results are similar to those to be reported by Rechtschaffen and Maron (in preparation) on the effect of amphetamine which reduced the amount of LVFS in human subjects. Gresham *et al.* (1963) found an insignificant increase in LVFS with 5 mg of amphetamine, but their data show a serial effect which was not entirely controlled by their design, and the results were inconsistent. Finally, the results of this study lend no support to the concept of a common physiological basis for dreaming and LSD-induced hallucinoid state.

SUMMARY

1. LSD in doses of 20 and 2 μg/kg interfered with sleep generally and reduced LVFS selectively in eight cats with chronically implanted electrodes.

2. The mechanism of the effect of this agent was not specifically elucidated; but it opposed whatever mechanism underlies SWS, and more clearly antagonized the system mediating LVFS.

3. An incidental finding was a progressive and marked increase in LVFS on successive days of an induced rest-activity cycle. Adaptation, fatigue, and/or sleep deprivation *per se* may have contributed to this phenomenon.

The author thanks Dr. Edward V. Evarts, in whose laboratory the work was performed, for advice throughout the study, and for his review of the manuscript.

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