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Lysergic Acid Diethylamide (LSD-25) Mimicks the Effect of Diffuse Light Input on EEG Correlates of Conditioned Operant Behavior in Cats

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Cats deprived of food and trained to press a lever for a milk reward, displayed postreinforcement EEG synchronization (PRS) associated with epicortical steady potential shift, i.e., "reward contingent positive variation" (RCPV) over the striate and parastriate cortex. Both phenomena depend on the input of diffuse light, even in subjects trained to perform in darkness. LSD-25 (15–30 μg/kg, im) restored the PRS–RCPV phenomena in total darkness. Pentobarbital (0.8–1 mg/kg, im) was ineffective although it significantly enhanced the PRS-RCPV responses in the presence of light. Some animals were more sensitive than others to LSD-25, and after administration of 20–30 μg/kg intramuscularly, showed dissociation between the EEG patterns and rewarded lever-pressing performance, which was particularly striking in the presence of ambient light.

Introduction

Cats deprived of food and trained to press a lever for 1 ml of milk reward, display bursts of high-amplitude 150- to 200-μv alpha activity of 5–9 cycle/sec (avg 7.5) over the primary and secondary visual projections. This phenomenon occurs during consumption of reward (4, 15, 20), and has been termed postreinforcement synchronization (PRS) (6, 23, 24); it is always associated with a transient surface-positive steady-potential shift of 200–400 μv over the same cortical region (16, 17). The positive shift, like the PRS, depends on the presence of light (17) and the taste of reward (6, 15, 16, 25). Therefore, it has been termed "reward contingent positive variation" (RCPV) (16, 18). The PRS–RCPV phenomenon does not depend on visual perception of reward and environment, since it also occurs in cats wearing translucent milky contact lenses that prevent patterned vision (17).

It is well known that LSD-25 causes visual hallucinations even in darkness. In its action on retinal ganglion cells this drug has a differential effect on their firing rate, i.e., an action which is similar to that of diffuse

light input; it markedly inhibits one population while stimulating another population of ganglion cells (see Discussion). The purpose of this study was to test whether LSD can substitute for diffuse light input during conditioned operant behavior and restore the light-dependent PRS–RCPV responses in total darkness.

The effect of LSD on PRS–RCPV responses was compared to that of a central nervous system depressant, pentobarbital, since there is substantial though indirect evidence that the PRS–RCPV phenomena result from transient but powerful suppression of the brain stem reticular activating system (4, 6, 15, 16, 18, 23–25). Hence, a moderate dose of pentobarbital may be expected to facilitate the occurrence of PRS–RCPV phenomena by shifting the dynamic balance between the internal excitatory (i.e., desynchronizing) and the internal inhibitory (i.e., synchronizing) systems toward the relative dominance of the latter.

Materials and Methods

Thirty experimental trials were carried out in four adult cats of either sex, trained to press a lever for 0.8 ml of milk reward presented on a schedule such that pressing a lever produced the reward aperiodically, approximately once every 4–7 sec. All subjects were kept on a 23-hr water- and food-deprivation schedule while being trained to press the lever until satiation, 5 days a week for 3–6 months. Three-fourths of the training sessions were conducted in the absence of light, and during the 4 weeks prior to this experimental paradigm, the subjects were allowed to perform only in the dark in order to induce extinction of possible conditional responses to light and perceived environment. Under pentobarbital anesthesia, three to ten solid-type, nonpolarizable Ag-AgCl electrodes (18) were implanted over the parieto-occipital cortex. Reference electrodes were implanted in the subjacent white matter 3 mm below the surface for transcortical recording which was found free from any discernible influences caused by directional changes in the standing corneo-retinal potential associated with eye movements (18). Lapping was monitored by using the milk delivery cup converted to a drinkometer (16). The technique of recording and integration of RCPV responses was previously described (18). The integrating system was calibrated to produce two pen deflections 30 mm each in response to a 100-μv positive shift lasting 1 sec. This value was arbitrarily accepted as one unit of RCPV triggered by 0.8 ml of milk reward.

During the control trials each subject was injected with saline solution (0.5 ml, im), and after 30 min was allowed to press the lever to obtain 40–60 milk rewards in the illuminated test chamber (the intensity of light was set at 26 cd/m^2), and, subsequently, 30–50 rewards in darkness. Immediately afterward, this sequence was repeated once, and the recording

session was terminated. The control sessions were alternated with the experimental ones on separate days, during which the cat was allowed to perform on the same schedule, but after administration of 20–30 μg/kg of LSD-25 or 1–2 mg/kg of sodium pentobarbital (Nembutal). Sixty per cent of the control and experimental trials were carried out in cats whose patterned vision was totally prevented by either a pair of translucent milky contact lenses or goggles in order to confirm our previous observations that diffuse light input but not patterned vision is essential in the full development of the PRS–RCPV responses (17). Statistical differences between the integrated average RCPV responses were determined by Student's t test.

Results

All subjects showed well-developed PRS–RCPV responses, but only in the presence of light, even though their patterned vision was prevented. Seventy-five to 80% of the mean integrated RCPV responses (each based on 10 single integrated RCPV) obtained while patterned vision was prevented were not statistically different from those obtained while patterned vision was allowed ($P > 0.05$) thus confirming our previous observations (17). The 20–25% decrease in the average RCPV responses during prevention of patterned vision was presumably caused by insufficient habituation to visual occluders, since this suppression of RCPV was always inversely related to the number of trials. A total 1900 consumptions of milk rewards in the dark were considered, and none of the subjects showed a single PRS–RCPV response, provided that all sources of light had been carefully eliminated. During a prolonged performance in the dark, usually a variant of PRS developed; it consisted of lower amplitude (70–120μv) and higher frequency (16–19 cycle/sec) spindle bursts which, however, were not associated with any consistent epicortical steady potential shift (Fig. 1, top).

"Light off" always abolished the PRS–RCPV responses not only during control trials (Fig. 1, top) but also in cats treated with Nembutal in doses of 1–2 mg/kg (not shown in Fig. 1), although this drug markedly enhanced the RCPV responses in the presence of light (see the averaged RCPV responses shown in Fig. 3). Higher doses of Nembutal were not used, since they interfered with lever-pressing performance. "Light on" usually promptly restored the PRS–RCPV responses after two to five rewarded lever presses. In several instances, the first rewarded lever press after "light on" restored the cortical responses, as shown in Fig. 1, top right.

In contrast to the control trials and to those after Nembutal, the animals treated with 20–30 μg/kg of LSD showed well-developed PRS–RCPV re-

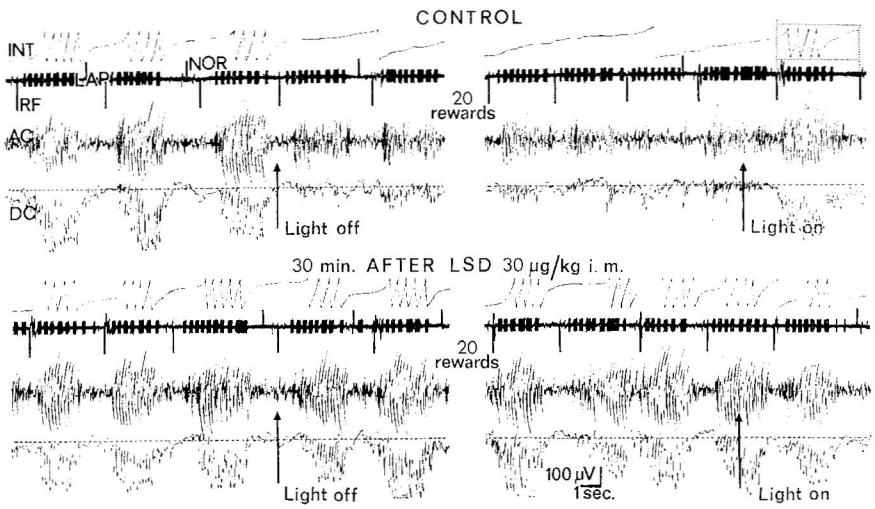

FIG. 1. The effect of LSD-25 on EEG patterns and steady potential shifts during performance in the presence and absence of light. Cat wearing translucent contact lenses that prevented patterned vision. Saline solution was administered (top) and LSD-25 (30 µg/kg, im) (bottom) 30 min prior to control and experimental session, respectively. Transcortical recording over the left posterior marginal gyrus with reference to subjacent white matter. Abbreviations: INT, integration of positive steady potential shifts during consumption of reward, i.e., RCPV responses; two upward deflections of the integrator equal to one RCPV unit, i.e., 100 µv positive steady potential shift lasting 1 sec. As shown in the last RCPV responses marked with dotted line (top right), the INT deflections were counted during the time period between two rewarded bar presses (RF). NOR, nonrewarded lever press. LAP, lapping recorded as bursts of 60 cycle/sec potentials recorded each time the subject's tongue touched the milk delivery cup. Note that the post-reinforcement sychronization (PRS) and the associated RCPV in the control experiment (top) were abolished during the light-off period, whereas after the administration of LSD well-developed PRS-RCPV responses were observed in darkness (bottom).

sponses during performance in complete darkness (Fig. 1, bottom). The averaged RCPV responses after LSD in the presence of light and in darkness are shown in Fig. 3.

Control RCPV responses and those observed in cats treated with Nembutal never occurred prior to or after nonrewarded lever press; they normally occurred 1–3 sec after the rewarded lever press, during consumption of reward, as judged by the contact of cat's tongue with the delivery cup (LAP in Fig. 1). On the other hand, in subjects treated with 20–30 µg/kg of LSD the PRS–RCPV responses often occurred prior to or after the nonrewarded lever press, in addition to responses observed during consumption of reward (Fig. 2, bottom).

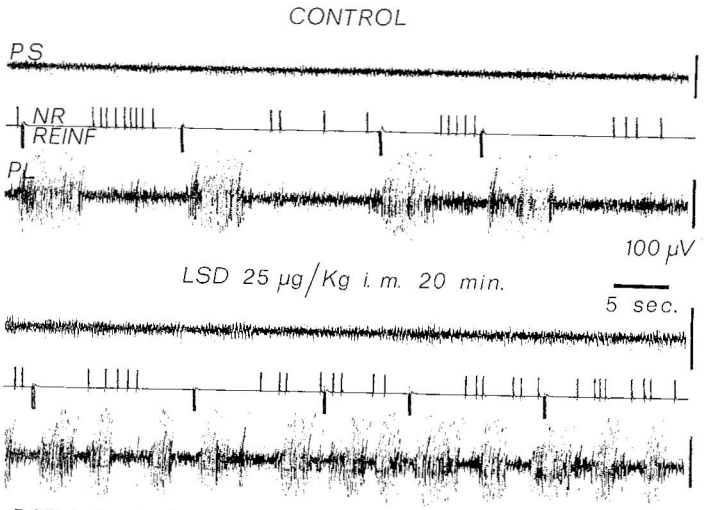

FIG. 2. LSD-induced dissociation between the EEG patterns and operant behavior. This subject was more sensitive to LSD than that used in experiment illustrated in Fig. 1: 30 min after administration of LSD (25 μg/kg, im) in the presence of ambient light high amplitude 7–9 cycle/sec responses resembling PRS were observed over the posterior lateral gyrus (PL) during non-rewarded (NR) and after rewarded lever press (bottom), whereas during the control experiment (top) the bursts of synchronized activity occurred only after rewarded lever press during consumption of reward; PS = posterior sigmoid gyrus. Recording with reference to the frontal sinus.

Similar to the well-timed postreinforcement EEG synchronization and steady potential shifts, the dissociated responses resembling PRS and RCPV occurred equally often in cats whose patterned vision was prevented. Approximately 80% of the dissociated responses were associated with the incorrect behavioral response, i.e., when the subject after a nonrewarded lever press licked the empty milk delivery cup.

The dissociation between the electrocortical responses and operant consumatory behavior was more pronounced in the presence of ambient light, and in two out of four cats; if expressed in number of RCPV responses per 100 nonrewarded lever presses, it ranged from 30 to 50% during the peak action of LSD administered in a dose of 30 μg/kg. Smaller doses of this drug, 15–20 μg/kg, caused the responses resembling PRS–RCPV to occur during approximately 20–30% of the nonrewarded lever presses. In the remaining two cats the dissociation was less pronounced after comparable doses of LSD: It ranged from 5 to 10% after 30 μg/kg of LSD. Higher doses of this drug than 30 μg/kg were not used since they interfered with lever pressing performance. Figure 3 summarizes the effect of

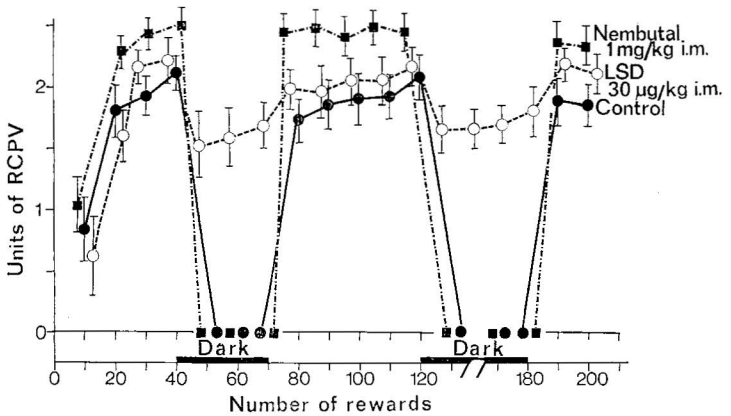

FIG. 3. Summary of the effect of Nembutal and LSD-25 on RCPV responses in the presence and absence of light. Data obtained in one cat (trained to perform while its patterned vision was prevented by translucent contact lenses) during three experimental sessions. Each session started 30 min after administration of: (a) Nembutal (1 mg/kg, im; filled squares); (b) LSD-25)30 µg/kg, im; empty circles); and (c) saline solution (control, filled circles). Each value (expressed in RCPV units) is the mean (with two standard errors) of 10 RCPV responses. Note that LSD restored the RCPV responses in total darkness, whereas Nembutal, although significantly enhanced the RCPV in the presence of light, failed to mimick the effect of diffuse light input.

Nembutal and LSD on average RCPV responses expressed in units of RCPV. Each average value (with standard error) is based on 10 measurements obtained in a cat wearing translucent contact lenses preventing patterned vision.

Discussion

In cats anesthetized with Nembutal, and in an unanesthetized midpontine preparation, focal steady illumination of the retina augments the over-all retinal discharge level recorded in the optic nerve, provided that the initial tonic discharge rate in darkness is relatively low (2). However, when the same intensity light is applied during a relatively high level of dark discharge rate, the over-all effect is a reduction of retinal output (2), since the simultaneous inhibition of the off-center ganglion cells weighs against the less pronounced on-center excitation (3).

In unanesthetized curarized cats the retinal on-center ganglion cells, with the increasing light intensity, increase their mean firing rate by up to 480% as compared to the tonic dark discharge level (3). Hence, in this animal preparation an over-all increase in the mass activity of the optic nerve can be expected to parallel the increasing luminance level, since the

enhancement of the firing of the on-center units is more pronounced than the inhibition of the off-center cells (3).

LSD in doses of 30–40 μg/kg administered intravenously markedly stimulates the discharge rate of two-thirds of the retinal ganglion cells, and inhibits the remaining one-third of the cells' population. These two effects have the same time course (19), and thus resemble the effect of a light stimulus. Relatively small doses of LSD, administered to unanesthetized cats, markedly augment the evoked potentials and afterdischarge oscillations to flash stimuli recorded in the lateral geniculate nucleus (9). Larger doses (160 μg/kg, iv) than those used by us partially inhibit synaptic transmission in the lateral geniculate nucleus of cat; this effect can also be obtained by a mild tetanizing electrical stimulation of the optic nerve (8). The last two observations indicate that smaller doses of LSD cause a subthreshold excitation of the retinal elements, whereas larger doses increase the gross output of the retina up to a level which may interfere with synaptic transmission in the lateral geniculate. This interpretation is supported by the following phenomena induced by the administration of LSD: enhancement of mass activity in the optic nerve (21); occurrence of spontaneous fluctuations in the electroretinogram (ERG) associated with bursts of electrocortical activity over the visual projections which can be abolished by section of the optic nerve (1); and augmentation of the light-induced ERG potentials which are particularly enhanced when submaximal flash stimuli are applied (5).

Nembutal, in a relatively small dose (5 mg/kg, iv) administered to unanesthetized cats, increases the tonic dark discharge of the retinal ganglion cells, enhances the inhibitory responses to steady illumination, and reverses the tonic augmentatory response to light into the inhibitory one (2, 7). The latter two phenomena indicate that Nembutal shifts the dynamic balance between the excitation and inhibition toward the latter. Hence, it is not surprising that this drug does not restore the PRS–RCPV phenomena in darkness, although it enhances these responses in the presence of light. The latter action, however, is presumably caused by a mild suppression of the arousal system and resulting preponderance of the synchronizing mechanisms.

We have suggested (17), on the basis of indirect evidence, that diffuse light input provides the energizing force necessary to trigger the recurrent or feed forward postsynaptic inhibition (or both) in neurons of striate and parastriate cortex, thus allowing a rapid transition from a low-voltage desynchronized pattern, characteristic of nonrewarded bar-pressing performance, to a synchronized pattern of PRS–RCPV responses during consumption of reward. This hypothesis is supported by the observation that the PRS–RCPV responses depend on the presence of light even in ani-

mals wearing translucent milky contact lenses (17). Electrical stimulation of the optic nerve in darkness during consumption of reward (but not during nonrewarded lever-pressing performance) substitutes for diffuse light input, and triggers, in a stimulus-bound manner, fully developed PRS–RCPV responses indistinguishable from those that occur spontaneously in the presence of light (Rick and Marczynski, unpublished results). Hence, it is suggested that LSD-induced retinal output mimicks that induced by diffuse light and thus provides the "electromotive" force to start the phasic recurrent inhibition on which the PRS–RCPV responses presumably depend.

The presence of on-units in the striate and parastriate cortex, i.e., those that show a sustained increase of discharge rate to a prolonged diffuse light input, is well documented (7, 10, 13). These cells, however, are greatly outnumbered by the on-off cells, i.e., those which respond only to a sudden change in the intensity of light stimulus, and those which respond only to a patterned, i.e., discrete and moving stimulus (11, 12). On-units characterized by an unusually regular maintained firing rate determined in an almost linear fashion by the intensity of light input, for this reason termed "luminance units," are also present in the retina of cat (3). In addition, photically sustained on-responses of units have been observed in the parvocellular layer of the lateral geniculate body, posterior hippocampal gyrus, and in the lateral tegmental process of the pons, beneath the trigeminal motor nucleus, in awake monkey (14). Their potential role in the effects of diffuse light input and in the pharmacological action of LSD on the PRS–RCPV responses remains to be investigated.

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