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LSD Acts Synergistically with Serotonin Depletion: Evidence from Behavioral Studies in Cats

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TRULSON, M. E. AND B. L. JACOBS. *LSD acts synergistically with serotonin depletion: evidence from behavioral studies in cats.* PHARMAC. BIOCHEM. BEHAV. 4(3) 231–234, 1976. – Administration of LSD (100 μg/kg, IP) or the serotonin depleting drug *p*-chlorophenylalanine (150 mg/kg/day for 5 days) both induce dramatic behavioral changes which include the common stereotyped responses of rapid flicking movements of the limbs and abortive attempts at grooming. The combined action of LSD and *p*-chlorophenylalanine results in a marked increase in the occurrence of these behaviors, suggesting that LSD acts synergistically with serotonin depletion. These data therefore support the hypothesis that the behavioral effects of LSD may be attributable to its well known electrophysiological effect of depressing the activity of serotonin containing neurons. In addition, limb flicking and abortive grooming may serve as a useful behavioral model for studying the actions of LSD, since these behaviors are quantifiable, easily scored, and occur with an extremely low frequency in normal cats.

LSD *p*-chlorophenylaline Serotonin Drug synergism Grooming behavior Hallucinations

LYSERGIC acid diethylamide (LSD), one of the most potent psychoactive agents known, has been the subject of intense experimental investigation, yet its mechanism of action is still a matter of debate. Recent evidence indicates, however, that LSD has a powerful depressant action on the discharge rate of serotonin containing neurons of the midbrain raphe nuclei [2]. Thus, the behavioral and perceptual effects of LSD may be attributable to the functional inactivation of the serotonin containing neurons in the brain. If this hypothesis is correct, LSD should act additively or synergistically with other treatments which inactive the brain serotonin system. Since previous studies have reported that the inhibition of serotonin synthesis, via the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine (PCPA), produces a stereotyped behavioral syndrome in the cat [7,12], we hypothesized that LSD would potentiate the behavioral effects of PCPA. Alternatively, the inhibition of serotonin synthesis following PCPA could be so complete as to render LSD ineffective, since the depression of neuronal activity in cells that have been previously depleted of neurotransmitter would be without further behavioral consequence.

In support of the former hypothesis, the present data indicate that LSD enhances, and in some instances produces, some of the signs of the behavioral syndrome which follows PCPA-induced serotonin depletion in the cat. The syndrome consists most conspicuously of rapid flicking movements of the forelimbs or hindlimbs, abortive attempts at grooming, hindlimb rigidity, ataxia, and swaying of the head.

METHOD

Eleven adult male and female cats were individually housed in stainless steel cages (18 × 32 × 24 in.) containing two horizontal perches. Behavioral observations were made in the cats' home cages during 1 hr periods. The behaviors emerging following LSD administration to PCPA-pretreated cats were determined in a pilot study and are defined as follows: (1) Abortive grooming: the cat orients to the body surface as if to groom, but does not emit the consummatory grooming response (bite, lick, or scratch), or emits the consummatory grooming response in midair; (2) Forepaw or hindpaw flicking: the cat rapidly flicks a fore or hindpaw as if shaking a foreign object such as water from the paw; (3) Hallucinatory-like behavior: the cat looks around at the floor, ceiling or walls of the cage and appears to be tracking objects visually, or the cat stares at the floor for long periods of time and occasionally hisses or swipes at unseen objects; (4) Swaying of the head; the cat's head moves back and forth approximately 1 cm horizontally at a

TABLE 1

FREQUENCY OF OCCURRENCE AND PRESENCE OR ABSENCE OF ABNORMAL BEHAVIORS FOLLOWING VARIOUS DRUG TREATMENTS

Treatment	N	Mean Hourly Occurrences + S.E.M. Abortive Grooming	Limb Flicks	Ataxia	Limb Rigidity	Head Swaying	Hallucinatory-Like Behavior
Saline	11	0.0	0.3±0.1	Absent	Absent	Absent	Absent
Amphetamine	11	0.0	0.2±0.1	Absent	Absent	Absent	Absent
LSD	11	4.2±0.1	8.3±0.4	Absent	Absent	Absent	Present
Saline+ PCPA	7	2.7±0.4	6.2±0.9	Present	Present	Present	Absent
Amphetamine +PCPA	7	2.1±0.3	9.6±1.2	Present	Present	Present	Absent
LSD+ PCPA	7	20.4±2.8*	39.7±4.4*	Present	Present	Present	Present

*$p<0.01$ for one-tailed t-tests comparing either the number of occurrences of abortive grooming or limb flicks for the LSD+ PCPA group with either LSD alone or the saline+ PCPA groups.

rate of about once per second. The abortive grooming and limb flicking behaviors were quantified by recording the number of occurrences of each behavior during the one hour observation periods. Each individual abortive grooming attempt or limb flick was counted as an occurrence, and consecutive occurrences of these 2 behaviors were usually separated by several seconds. The head swaying and hallucinatory-like behavior were scored as present or absent. In addition, the presence of ataxia and hindlimb rigidity were assessed at the completion of each one hour observation period by placing the cat on the floor of the room for 10 min and observing stiffness of limbs while walking and the degree of ataxia or loss of balance.

Behavioral observations during a 1 hr period were made on all cats following saline administration (2 ml, IP). Immediately following this, half the cats received LSD-25-tartrate (100 μg/kg, IP, dose calculated as salt), and the other half received *d*-amphetamine sulfate (1 mg/kg, IP, dose calculated as salt), and behavioral observations during a 1 hr period were again taken. On the following day, saline baseline data was again obtained and then the cats that received LSD on the previous day received amphetamine, and vice versa. Seven of the cats then received daily injections of PCPA (methyl ester HCL, 150 mg/kg/day, IP, dose calculated as salt) for 5 consecutive days and 4 cats received daily injections of vehicle (saline, 2 ml, IP) for 5 consecutive days. On Day 6, behavioral observations were taken on all cats following saline administration (2 ml, IP), and the cats in each of the 2 groups (PCPA and vehicle) were then divided into 2 subgroups. The cats in one subgroup received LSD (100 μg/kg, IP) and those in the other subgroup received amphetamine (1 mg/kg, IP) and behavioral observations were again taken. On the following day, saline baseline data were obtained and the LSD and amphetamine treatments were counterbalanced, as described above, and the final 1 hr period of behavioral observations was obtained. The cats were then deeply anesthetized with sodium pentobarbital (35 mg/kg, IP), and the brains were removed and assayed for serotonin content, using a modification [11] of the method of Bogdanski *et al.* [5].

RESULTS

Table 1 summarizes the behavioral data for all treatments. Normal home cage behavior was observed after saline administration, with the cats spending much of their time quiescent or sleeping, and most of their remaining time grooming, eating, and drinking. None of the cats displayed any abortive grooming behavior and only one cat displayed paw flicking (0.3 ± 0.1 mean hourly occurrences) after saline administration. Following the administration of amphetamine (1 mg/kg), the cats remained relatively inactive, displayed no dramatic behavioral changes, but showed increased eye movements. In previous investigations, this dose of amphetamine also produced little overt behavioral change, but the continual movement of the eyes and a tonically desynchronized EEG indicated that the cats were in a mildly aroused state (Jacobs, unpublished observations). Administration of LSD (100 μg/kg) produced what appeared to be prominent hallucinatory-like behavior in which the cat continually scanned the cage, often stopping and staring for long periods, and made pawing movements at unseen objects. LSD also induced abortive grooming behavior (4.2 ± 0.1 mean hourly occurrences) and paw flicking (8.3 ± 0.4 mean hourly occurrences). By Day 5, daily administration of PCPA (150 mg/kg/day) produced ataxia, limb rigidity, and head swaying, as well as abortive grooming (2.7 ± 0.4 mean hourly occurrences) and paw flicking (6.2 ± 0.9 mean hourly occurrences). The abortive grooming and paw flicking behaviors appeared qualitatively identical before and after PCPA administration. Administration of saline to PCPA pretreated cats produced no change in their behavior, while LSD administration produced a substantial increase in the amount of abortive grooming behavior (20.4 ± 2.8 mean hourly occurrences) and paw flicking (39.7 ± 4.4 mean hourly occurrences). Other behaviors occurring after administration of PCPA or LSD included kneading of the forepaws, meowing, head shaking, and rubbing against objects. None of these latter behaviors were markedly enhanced by a combined treatment of PCPA and LSD. No discernible changes in the degree of ataxia, limb rigidity, or head swaying were

observed following LSD administration to PCPA pretreated cats. While difficult to quantify, the degree of LSD-induced hallucinatory behavior seemed to be enhanced by PCPA pretreatment. Administration of amphetamine to PCPA pretreated cats did not increase the frequency of abortive grooming or paw flicking behaviors ($p>0.05$, two-tailed t test), indicating that the sharp increase in the abnormal behaviors following LSD administration to PCPA pretreated cats was not due to a general arousal effect. The level of whole brain (minus cerebellum) serotonin in PCPA treated cats was significantly decreased by 86%, as compared to the control cats (0.032 ± 0.004 μg/g vs. 0.233 ± 0.011 μg/g, $p<0.01$, t test).

In summary, certain abnormal behaviors, i.e., paw flicking and abortive grooming behavior, emerge after either LSD or PCPA administration, and the combined action of LSD and PCPA results in a marked increase in the occurrence of these behaviors.

DISCUSSION

The types of behaviors produced by PCPA and LSD may be divided into 3 categories: hallucinatory-like behavior, motor disturbances, and abnormalities in grooming behavior. A mechanism has been proposed to account for the perceptual disturbances occurring after LSD administration. This mechanism is based on the finding that serotonin has an inhibitory effect on its postsynaptic target neurons [8]. LSD inhibits raphe neuronal activity, leading to reduced release of the inhibitory transmitter and hence an increase in the activity of neurons postsynaptic to raphe neurons [8]. The prominent connections of serotonin containing neurons with neurons in the visual system, and the disinhibition which follows the administration of LSD, may account for the visual hallucinations. The ataxia, limb rigidity, and head swaying observed after PCPA and LSD may be due to a loss of the serotonergic influence on motor systems which may be mediated by prominent descending serotonergic projections into the spinal cord, which terminate on or near alpha motoneurons [6]. The flicking of the limbs observed in the present study is a naturally occurring grooming response used to remove foreign objects from the limbs, and is abnormal when it occurs in the absence of tactile stimulation to the limbs. The abortive grooming behavior observed after LSD and PCPA consists of the appetitive or orienting components of grooming behavior without the consummatory responses that normally follow the body orientation. When the consummatory grooming responses did occur, they were usually poorly directed, i.e., they frequently were not directed toward the body surface. The abortive grooming behavior and paw flicking may be due to decreased serotonergic influences on motor systems (see above) and/or to changes in tactile sensitivity. Several studies have reported that serotonin depletion affects at least one kind of somatosensory sensitivity (e.g. [9,10]).

The present data indicate that LSD acts synergistically with serotonin depletion since a combination of LSD and PCPA produced a marked increase in the occurrence of several of the behavioral effects of either LSD or serotonin depletion alone. The alternative hypothesis that LSD would be ineffective in PCPA pretreated cats was not supported. The inhibition of neuronal activity by LSD in cells that have been depleted of their neurotransmitter might be expected to produce no further behavioral consequences. However, PCPA in the dosages used did not totally deplete serotonin, and thus some functional synaptic activity may remain after PCPA treatment. Similarly, LSD only partially inhibits the activity of serotonergic neurons in freely moving cats [13], and induces only a few of the abnormal behaviors. When LSD is administered in combination with PCPA, not only is the amount of neurotransmitter greatly reduced, but, in addition, neuronal activity is inhibited [14]. Under these circumstances, the abnormal behavioral syndrome emerges in its most prominent form.

Previous studies investigating the combined effects of LSD and serotonin depletion on behavior reported that PCPA potentiated LSD's effect of disrupting a bar pressing response in rats [4]. This disruption was attributed to a postsynaptic supersensitivity effect since at that time it was believed that the primary action of LSD was the stimulation of postsynaptic serotonergic receptors in the brain [1,3]. However, more recent research has revealed that LSD has very potent presynaptic inhibitory effects on raphe neurons, but little, if any, postsynaptic effect in low doses [8]. Furthermore, recent studies from our laboratory have failed to provide evidence for PCPA induced supersensitivity [15]. Although Appel *et al.* [4] showed that LSD and PCPA were additive in disrupting bar pressing, there is no reason to believe that this effect was mediated by the same mechanism, i.e. that the drugs acted synergistically. However, the fact that LSD and PCPA both elicited and/or potentiated behaviors with a very low frequency of spontaneous occurrence, such as limb flicking and abortive grooming, is more supportive of the hypothesis that LSD and PCPA act synergistically.

In conclusion, the present data support the hypothesis that the behavioral effects of LSD may be attributable to its well known electrophysiological effect of depressing the activity of serotonin containing neurons in the CNS. In addition, limb flicking and abortive grooming may serve as a useful behavioral model for studying the actions of LSD, since these behaviors are quantifiable, easily scored, and occur with a very low frequency in normal cats.

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